SEX CONTROL AND KNOWN CORRELATIONS IN PIGEONS[1]

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WHEN one nowadays states that he has obtained a real control—a reversal—of the development of sex, he can feel assured that his biological audience demands a very large volume of rigid proofs. The first reason for this large requirement is, as you well know, that the assertion of sex control has been often made, and that in most of these cases the data have proved disappointing; inadequate in one or another respect. A second reason for present widespread skepticism as to even the possibility of a real control of sex-development centers in the now well-demonstrated fact that in some groups of animals, the male—and in other cases the female—produces sex cells of two kinds when these are considered from the standpoint of their chromosomal numbers or characteristics; and further that each of these two groups of germs *normally* gives rise to organisms of the sex corresponding to the chromosomal constitution of these germs. Moreover, certain linkage phenomena observed in breeding such forms, unquestionably show themselves to be *normally* associated with these same chromosomal differences.

But the experimentalist has learned through some pre-

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vious contests with ideas of fixity and causality that when *normal* structural correlations have been demonstrated in the field of development, *nothing* has been decided as to *causality* and *inflexibility;* indeed it is commonly at such a point that experiment applies the pressure of *new* or *unusual* conditions and makes an approach toward learning the nature of a phenomenon, by forcing the latter to break from its normal correlations, and disclose something of its real nature through its versatility—through its own capacity to shift from response to one set of conditions, to response to another set of conditions. Laws of causation, in the field of development, are not to be deduced from studies concerning the normal associations of the *structures* of the cell; they may be approached through demonstrations of the versatility and responsiveness under pressure of those *processes* native to living matter.

We have stated that when sex is controlled an audience like this will demand a volume of proof. It is clear that the time limits here do not admit so extensive a presentation. I should like to note here, however, that Professor Whitman's complete studies on sex in doves and pigeons have been prepared for publication, and for several months have been in the hands of the publisher. The results of my own studies of the past five years designed to test the reality of the sex-control, and the nature of sex, as exhibited in these forms, will already doubtless fill another volume. And, since the last volume of the posthumous works of Professor Whitman is nearing completion, I can promise that it will not be long before the work of preparing my own results for publication will be begun. Only when all of these data are fully available to you, may we expect a judgment as to whether the evidence for our thesis to-day is adequate. It is possible to give here, within the time limits, only an outline of the *kinds of study* which have yielded evidence on the question of sex-control in pigeons.

These studies were begun, and carried on for many years by Professor Whitman. He obtained indispu-

tably—a profound modification of the sex-ratio, and identified in a general way the factors associated with the modified ratios. Whether the modified ratios signified a real control—a reversal—of sex could not at that time be definitely decided. It was to help in making a decision as to whether the changed sex-ratios signified a real—or only an apparent—reversal of sex that I proposed in the winter of 1908–9 to carry out some chemical studies on the ova of the doves and pigeons which in Whitman's hands were yielding these striking sex ratios. The methods for the quantitative and qualitative analysis, of the very small samples to be used, were developed, and these were tested during 1909–10 on considerable numbers of the larger ova of jungle fowls and domestic fowls. Since April, 1911, I have carried on this and other lines of study to determine if possible whether the changed ratios observed by Whitman involve a real reversal of sex; this work is being actively continued.

Whitman showed that "width of cross" in doves and pigeons is of first importance in determining sex ratios and that the wider the cross the higher is the proportion of males. Family crosses produce—in practically all matings—only male offspring. Generic crosses produce from their "stronger" germs—those of spring and early summer—nearly all males. If, however, the birds of such a generic cross be made to "overwork at egg-production"—that is if their eggs are taken from them as soon as laid and given to other birds for incubation— then the same parents which in the spring threw all or nearly all male offspring may be made to produce all, or nearly all, female offspring in late summer and autumn. At the extreme end of the season eggs capable of little, then of no development, are often found in such a series. As the birds of such a mating grow older the time of appearance of females, and of eggs incapable of full development, is reached earlier and earlier in the summer or spring.

In the case of a number of hybrids Whitman showed that *color* is also affected by this pressure of reproduc-

tive overwork and season. White color could be obtained from the later, "weaker" germs, though this color did not appear in birds from the "stronger" germs of the earlier season. And further, that white, or whitened, "mutants" from pure breds were derived almost or quite exclusively from those conditions which produce "weakened germs." Among such conditions are late season and overwork, inbreeding and great extremes of age—either very old or very young. This brief outline of Whitman's findings on sex is perhaps a more adequate, and more accurate, one than I was able to give to one of the societies represented here when I had only begun the examination of this data in 1911. Two brief summaries given on the chart (not given here) will assist in obtaining a picture of the nature of the results. I may add that by very strongly "overworking" females of some species —overworking them more strongly than Whitman did— I have been able to obtain a high predominance of females during autumn from a cross merely of *specific*[2] value. This result is illustrated by Chart II, though the matings there exhibited were prepared for the primary purpose of illustrating results in the study of size. It will be noted in the chart that parents overworked in a previous year throw a high proportion of females during the whole of the succeeding year, and most markedly in late autumn. In this mating the ratio at the end of the season is 14 females to 1 male; in the other (not previously overworked) there was an excess of females only after overwork—during the latter half of 1914 ($7\male$:$10\female$); and in the year following this overwork there were 21 or more females, to 11 or fewer males. Such data are not exceptional; they coincide with the usual.

Now, in the generic crosses which give all, or nearly all, males at the beginning of the season and all, or nearly all, females in the autumn what is happening?—true sex reversal? or is it selective fertilization, differential mat-

[2] Some data from pure breds (pure species) mated to their own kind show also this predominance of females from late autumn under extreme overwork; such predominance is here probably less pronounced than in the case of the crosses.

uration or a selective elimination of ova in the ovary? This was from the first the whole of our own problem. We have had no other, nor have we now, except in so far as the entire question of the *nature* of sex—in germ and adult—is concerned.

Our method has been to study the eggs, progeny and parents of such series as show this seasonal "reversal of the dominance" of sex from as many different angles as possible. The result till now is that we have learned some ten kinds of facts concerning the germs, or the prospective value of the germs, which issue from such a series. Let us note that these ten lines of correlated fact do not relate merely to a "normal" state of the germs, but have to do with measurable changes which occur when ova are subjected to the stress of parental reproductive overwork, which as Whitman has shown is accompanied by a shifting from male-production to female-production during the progress of the season. The diagrams of chart I will assist in making clear the nature and significance of the several correlations. The solid lines indicate a *double* correlation, *i. e.*, for both season and egg of clutch; the broken lines represent correlations established thus far for only one of these.

The generic cross that has been most fully studied involves *Turtur orientalis*—the Japanese turtle dove, and *Streptopelia alba*—the white ring dove. These species together with their reciprocal hybrids are shown (photographed) in another chart (not given here). Some data for egg size, and for sex-differences in the adult size of the several forms concerned—parents and reciprocal hybrids—are also given in that chart.

The first correlation that we have established for this series results from a study of the size of ova—*i. e.*, of yolks freed from shell and albumen. The result clearly establishes the fact that the yolks of late summer and autumn—those that produce mostly, or all, females—are larger than the yolks produced in the spring which give rise to males. And there is no jump from the one size to the other, but what may be better described as a

gradual enlargement.   This fact is represented diagrammatically on chart I, and some of the actual figures may be had from the charts dealing with size of offspring (II), with analyses (III), and with calorimetry (IV).
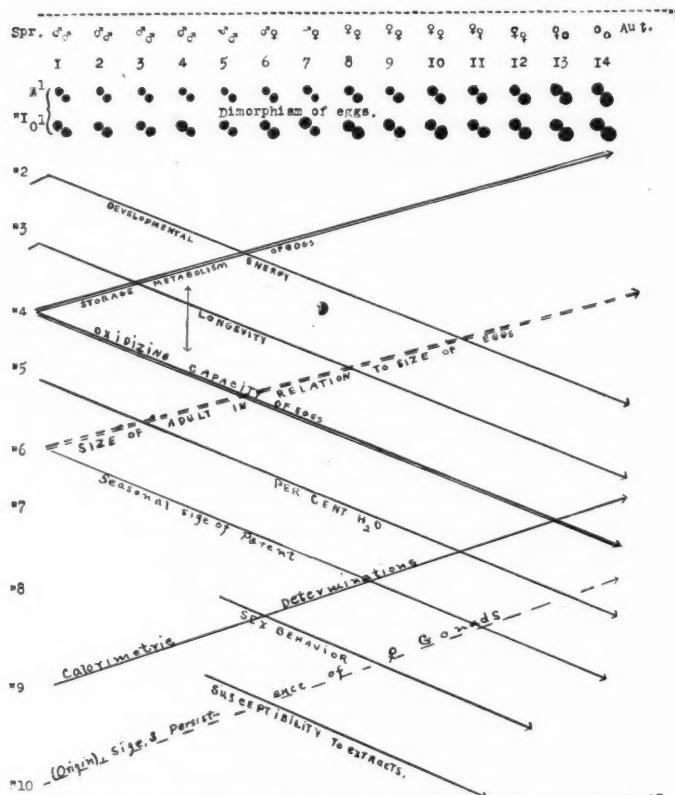

CHART I

COMPARATIVE SIZE OF EGGS OF ALBA (*a*) AND ORIENTALIS (*o*).

At the same time that this seasonal increase in size of yolks was learned, it also became evident that the eggs of doves and pigeons are *dimorphic*: That the two eggs forming the pigeon's clutch bear usually—there are exceptions—a smaller yolk in the first, and a larger yolk in the second.   Since Whitman had already shown that

in the pure wild species with which he worked, males predominate in hatches from first eggs of the clutch, and females predominate in hatches from second eggs of the clutch, it became evident that the male-producing yolk is smaller—both in relation to season, and to egg of clutch, than is the female-producing yolk. Corresponding to the fact (commonly obtained from matings of individuals of the same species) that two males or two females may sometimes arise from the same clutch, we have found that a similar number of pairs of yolks of these forms are equal in size; and too that such pairs may be either large or small. The charts just referred to may be consulted in this connection. We have previously noted (1911, 1912) that in eggs laid by hybrids neither sex nor yolk-size bears the above described relations to the order of eggs in the clutch.

Still a third situation has yielded positive evidence that the smaller yolks are male-producing and the larger yolks female-producing—namely that in respect to age. It has already been mentioned that Whitman learned that the females which were "overworked" tended, when older, to begin the production of females at earlier and earlier stages of the season. Now a comparison of the size of yolks derived from younger and from older birds has conclusively shown smaller eggs for mature but younger birds, as compared with the old birds (see Chart 2). In scores of individual cases the yolk-size has now been followed from youth, and comparative youth, to old age.

In even a fourth situation it has been possible to test the relation of yolk-size to sex. Breeding data show that from the very first egg in life, and the very first egg produced after a long period of rest or inactivity, more frequently produce a *female* than do the first eggs of succeeding pairs, or clutches. Our studies on the size of such yolks show a wholly similar reversal of order of size of the two eggs of the very first clutches; the size reversals here being more frequent than in the succeeding clutches (see Chart 4).

CHART II

BREEDING RECORDS—1914

♀ *St. risoria 641 (old)*; *1913 = 42 eggs*

Series 1

♀ A1	1/1	White 140		♀ T1	7/20	White 140
♀ A2	1/3	White dead 2/3		♂ T2	7/22	Dark 164
1st (4) = 2.066 g.		2d (4) = 2.243 g.				
				♀ U1	7/28	White 144
H1	4/4	Inf. yolk = 1.995 g.		♀ U2	7/30	White 151
H2	4/6	Inf. yolk = 2.105 g.				
				♀ V1	8/14	White 155
♀ I1	4/12 ⎱	⎰ White killed 4/29		♂ V2	8/16	Dark 169
♀ I2	4/14 ⎰	⎱ White 158 (?2)				
				♀ W1	8/22	White 152
♂ J1	4/21	Dark killed 2/25		W2	8/24	Soft at pole
♀ J2	4/23	White 158				
				♀ X1	8/30	White 161
♀ K1	4/29	White 147		♀ X2	9/1	White 145
♀ K2	5/1	White 151				
				♂ Y1	9/9	Dark 161
L1	5/9	Broken		♀ Y2	9/11	White killed
L2	5/11	Dark				
				♀ Z1	9/18	White ——
♂ M1	5/18	Dark 161 (?1)		♀ Z2	9/20	White dead 10/26
♀ M2	5/20	White 163				
				♀ AA1	9/26	White 141
♀ N1	5/30	White 150		♀ AA2	9/28	White 146
♀ N2	6/1	White killed with ext.				
				♀ BB1	10/7	White 150
				♀ BB2	10/9	White 144
♂ O1	6/7	Dark 150				
♀ O2	6/9	White 150		? ♀ CC1	10/17	Dark dead 11/8
				♀ CC2	10/19	White dead 11/10
♂ P1	8/18	Dark 149				
P2	6/20	Broken		♀ DD1 ⎱	10/26	White 130 (?1)
				♀ DD2 ⎰	10/28	White 162 (?2)
♀ Q1	6/28	White 143				
♀ Q2	6/30	White 137		♂ EE1	11/6	Dark 152
				♀ EE2	11/8	White 143
♀ R1	7/4	White 154				
♂ R2	7/6	Dark 162		♀ FF1	11/16	White 166
				FF2	11/18	Broken
? ♂ S1	7/12	Dark dead 7/29				
♀ S2	7/14	White dead 7/31		♀ GG	11/26	White 150

1st 16 = 5♂ : 11 ♀   2d 15 = 5♂ : 10 ♀   last 15 = 1♂ : 14 ♀

The relation of the order of the eggs in the clutch to the prospective sex of the offspring is an important point, and we wish here to make this situation clear, since it seems that two rather brief statements made in 1911 and 1912, before the Society of Zoologists, have not been understood by all.

From the time of Aristotle to the present year there

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CHART II—*Continued*

BREEDING RECORDS—1914

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♀ *St. risoria 647 (young)*; *1913* = *18 eggs*

Series 2

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C1	2/8	Inf. yolk = 1.445 g.	♀ P1	7/1	White 150	
C2	2/10	Broken	♀ P2	7/3	White 15 da. emb.	
♂ D1	3/5	Dark embr.	♀ Q1	7/9	White 148	
♀ D2	3/7	White	♂ Q2	7/11	Dark 164	
♂ E1	3/19	Dark 167	♀ R1	7/22	White 152	
♂ E2	3/21	Dark 180	♂ R2	7/24	Dark 172	
♀ F1	3/29	White 154	S1	8/3	White 13 da. emb.	
♂ F2	3/31	Dark 190	S2	8/5	Broken 3 da. emb.	
♂ G1	4/8	Dark killed 5/6	♂ T1	8/12	Dark 174	
♀ G2	4/10	White killed 5/3	♀ T2	8/14	White 164	
♀ H1	4/16	White 153	U	8/20	yolk = 1.490 g.	
♀ H2	4/18	White 153	V1	9/6	"Blood circle"	
♂ I1	4/25	Dark 169	♂ V2	9/8	Dark 170	
♀ I2	4/27	White 154	?W1	9/19	Dark dead 10/16	
J1	5/5	3-da. embr. killed	♀ W2	9/21	White dead 10/14	
J2	5/7	3-da. embr. killed	♂ X1	9/30	Dark dead 10/19	
♂ K1	5/14	Dark 169	♀ X2	10/2	White 145	
♀ K2	5/16	White 158	Y1	10/29	Inf. yolk = 1.845	
♂ L1	5/25	Dark 179	♀ Y2	10/31	White 15 da. embr.	
♀ L2	5/27	White 164	Z1	12/27	No dev. yolk = 1.870 g.	
♂ M1	6/3	Dark 169	Z2	12/29	No dev. yolk = 1.925 g.	
♀ M2	6/5	White 11 da. emb.	♀ 641 = (170) (♂ 170)			
♂ N1	6/13	Dark 165	♂'s (5) from 1st = 155		♀'s (13) = 149	
♀ N2	6/15	White 150	♂'s (3) from 2d = 165		♀'s (11) = 150	
♂ O1	6/22	Dark killed 7/13	♀ 647 = (166) (♂ 165)			
O2	6/24	Broken	♂'s (7) from 1st = 170		♀'s (5) = 151	
			♂'s (5) from 2d = 175		♀'s (6) = 158	

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1st 17 = 9♂ : 8♀   2d 17 = 7♂ : 10♀   *1915* = 11 dark : 21 white

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have appeared statements concerning a predominance—
or a lack of predominance—of males from the first egg
and of females from the second egg of the pigeon's clutch.
It is unnecessary to outline these divergent reports. It
is only necessary to point out the reason for discordance;
though the reason we had thought to be quite obvious
since 1911. The statements hitherto made have all been
based on a general statistical method, which is a wholly

CHART III

SUMMARY OF PARALLEL BREEDING AND CHEMICAL STUDIES ON THE EGGS OF
♀ *T. orientalis* No. 500X *St. alba* No. 410 FOR THE YEAR 1912

Date	An'l's or Inc.	Wt. of Yolk	Result						
			Alc. Soluble	Phos- phatids	Protein	Ext.	Ash	H₂O	Energy Total
4/13			Broken when found						
4/15			Broken when found						
5/26	159	2.330	72.65	18.32	25.44	5.28	4.85	57.01	7,405
5/28	160	2.660	72.45	17.54	25.63	5.25	2.62	54.82	8,990
6/7	Inc.	....	Only one egg laid						Dark ♂
6/15	Inc.	....	....	....	....	....	....	....	Dark ♂
6/17	Inc.	....	"Very large egg"			....	....	....	White ♀
6/24	Inc.	....	....	....	....	....	....	....	No. dev.
6/26	Inc.	....	....	....	....	....	....	....	Dark ♂
7/3	186	2.026	72.21	16.49	26.00	3.63	2.43	56.05	6,714
7/5	187	2.330	72.27	19.18	26.55	3.75	1.93	55.22	7,881
7/15	Inc.	....	....	....	....	....	....	....	Dark ♂
7/17	Inc.	....	....	....	....	....	....	....	Dark ♂
7/23	192	2.422	72.42	17.82	25.88	3.82	1.80	55.84	8,061
7/25	193	2.720	72.45	18.88	25.96	3.86	1.81	55.33	9,296
8/2	Inc.	....	....	....	....	....	....	....	Dark ♂
8/4	Inc.	....	....	....	....	....	....	....	Dark ♂
8/13	Inc.	....	....	....	....	....	....	....	No. dev.
8/15	Inc.	....	....	....	....	....	....	....	Dark ♂
8/23	Inc.	....	....	....	....	....	....	....	No dev.
8/25	Inc.	....	....	....	....	....	....	....	White ♀
9/15	Inc.	....	....	....	....	....	....	....	White ♀
9/17	Inc.	....	....	....	....	....	....	....	White ♀
11/29	259	2.700	73.17	21.40	25.23*	....	....	55.52	9,323
12/1	260	2.715	73.02	21.63	25.38*	....	....	55.39	9,383

* Calculated.

inadequate and useless one for a study of the problem.
It is now clear that the method that would be valuable for
this purpose must be a thoroughly *analytical* one. Whit-
man has properly analyzed this situation. He has shown
that normally—*i. e.*, with effects of *crossing* eliminated—
from the periods for the production of the strongest
germs an undue proportion of *pairs* of eggs produce
males; and from the opposite period there arise undue

CHART IV

STORED ENERGY OF EGGS (1914) OF *Streptopelia risoria* (558) AS DETERMINED BY THE BOMB CALORIMETER

No.	Date	Wt. Yolk	Energy	Per Cent. Diff.
665	A1　6/6	1.010[1]	3,358[1]	
666	A2　6/8	0.970	3,175	−5.8[2]
674	B1　6/19	0.855	2,807	
675	B2　6/21	1.000	3,245	+15.6
699	C1　7/14	1.145	3,815?	
700	C2　7/16	1.463	5,008	+31.3?
728	D　8/30	1.395	4,812	
——	E　9/9 or 10 soft shell, broken			
. . .	F1 10/17　"　"　"			
. . .	F2 10/19　"　"　"			
770	G1 11/6	1.440	4,837 (?)	
771	G2 11/8	1.720	5,797	+19.8 ?
774	H1 11/20	1.590 +sl. loss	4,906 +	
775	H2 11/22	1.780	6,015	+22.6 −
776	I1　12/1	1.640	5,614	
777	I2　12/3	1.820	6,255	+11.4
781	J1 12/12	1.535	5,302	
782	J2 12/14	1.690	5,601	+5.6
791	K1 12/23	1.485	5,266 (?)	
792	K2 12/25	1.718	5,880	+11.7 ?

[1] This egg was not only the first laid during season, but first during life of this bird.

[2] The percentage differences are based upon a value of 100 per cent. for the smaller egg of the pair.

numbers of pairs of eggs that produce females. To lump these all together and to count the number of males arising from first, and females from second eggs is plainly to cover up or to lose the significance of the intervening pairs of eggs which bear the significant data. Again, many matings, because of exceptional strength or of weakness, will yield a considerable total predominance of males or of females, and the statistical method lumps all these and others without thought or care of the cancellations and unsatisfied cancels involved; all of which as easily contributes to a *smoothing* of the results, as it does to a *smothering* of them.

But Whitman has also shown that not only is the *method* previously employed at fault, but that, much more important still, the *material* used—in probably all of those cases in which no correspondence of sex to the

order of the eggs of the clutch was found, and where the
worker has thought it worth while to mention the *kind*
of birds studied—such material has been wholly unsuit-
able to leading to a decision. That is to say, the
"pigeons" used in these cases were one or another of the
150 *mongrels* collectively known as domestic pigeons.
One of the clearest points of our present knowledge of
the relation of sex to egg of clutch is that the normal re-
lations are lost *immediately upon hybridization—i. e.,* in
passing from the *pure state of the species.* The count-
less degradations and crossings suffered by the various
domesticated breeds since their existence as a pure
species, is therefore a sufficient index of the suitability
of this material for a study of this subject. Whitman
demonstrated the predominance of males from the first,
and of females from the second egg of the clutch when
pure species mated with pure species produce the eggs,
and also the random distribution of the sexes from the
eggs of hybrids. And as early as 1911 and 1912 I dem-
onstrated charts and lantern slides which showed that the
size of the yolks from pure species showed with consid-
erable uniformity a smaller first, and a larger second
yolk; and further, that this regularity breaks down at
once and completely in hybrids.[3]

Let us now note the conclusions which follow upon the
demonstrated dimorphism of the ova[4] in the pigeons,
when this is reviewed in the light of breeding data on
these forms and in connection with the demonstrated re-
lationships of size of yolk to sex—relationships which
are continued even under the pressure brought by over-
work, season, and age.

It becomes clear, first of all, that a selective fertiliza-
tion by one kind of sperm is quite impossible—the sex

[3] Note that in Chart 2, already referred to, where the eggs are produced
by the female cage or blond ring dove—in which *purity* of the species is
often doubtful—that a predominance of males from first, and of females
from the second egg of the clutch is indicated in both series. In series I,
where the two sexes arise from a single clutch, the first egg gave rise to
the male in 6 (or 7?) cases; to a female in 3 cases. In series II the first
egg yielded males in 9 (or ?10) cases; females in only three cases.

[4] Yolk size has now been accurately determined in about 10,000 cases.

differential residing in two kinds of eggs and not in the sperm. We may here recall that previous to our own studies, breeding data obtained from other birds had indicated that in the birds the sexually dimorphic germs are borne by the female—or to use Mendelian terms, that the female bird is heterozygous for sex.

The second conclusion that must be drawn is that a selective elimination of ova in the ovary does not occur during "overwork," while mated to a mate of another genus, nor otherwise, since the two kinds of ova are—from their size relations—positively known to present themselves under these, and under all the conditions which have been studied. In other words, the generic cross, which produces all or nearly all males in the spring, and all or nearly all females in the autumn, is utilizing in the spring a number of female-producing ova for the production of males, and in the later season is utilizing for the production of females ova one half of which had initial inclinations for the production of males. Note too that the evidence for the continued production during the season of ova of two kinds as regards sex does not rest alone on our knowledge of the dimorphic ova. For, from breeding data we learn that if the *same female* which threw all males in the spring and all females in the autumn, had been mated to one of her *own* species, then both males and females would certainly have appeared at all seasons, and largely or wholly in relation to the order of the eggs of the clutch, with but slighter effects of season to be noted. If the overwork were extreme, a predominance of females in late autumn might be expected; but in the earlier season the sexes would surely be found in nearly equal numbers. Several of the correlations soon to be mentioned, moreover, further attest that ova of two grades—in respect to sex—are produced throughout the year.

The data thus far examined exclude the possibilities of accounting for the observed sex-ratios of the generic cross on the basis of a selective action of the sperm, or of a selective elimination of ova in the ovary. What light do these data shed on the possibility of accounting

for the seasonal difference in sex-production on the basis
of a differential maturation? The fact that the sperm is
present in the pigeon's egg during the whole of the
second maturation division may properly raise this ques-
tion. On this point we must say that the particular data
we have just been citing are perhaps not entirely con-
clusive; these data alone, however, offer the following
significant points for consideration: To account for the
observed sex ratios of the *generic cross* the maturation
would have to be definitely differential in (1) the elim-
ination of an X chromosome[5] during the spring from one
half of the ova, and the retention of this same X in the
homologous[6] eggs of the autumn. (2) The elimination
of a Y chromosome from the other half of the eggs laid
during the autumn, and the retention of all these same
Y's in homologous eggs of the spring; and (3) all other
chromosomes than the sex chromosomes must display
no such thing as seasonal preferences for "staying" or
for "going," since every observable character of the
hybrids betrays the presence of both of the parental
genera. This is not all, but let us pause at this point to
note that even if the sex chromosomes were here capable
of such wholly unknown and almost unthinkable be-
havior, that they have—after all—in this case wholly
lost the *initiative* in governing sex, since it is the *place*
in the season and the *degree* of the *pressure* of the *over-
work* that has been shown to prescribe the sex of the off-
spring; and further, the correlations of size, water con-
tent, energy storage, etc., which we have proved to exist
throughout the whole season—these correlations are all
established *prior to* the formation of even the first polar
body; this latter being formed only at the time of ovula-
tion, and the second polar body forming 1 to $1\frac{1}{2}$ hours
after the entrance of the egg into the oviduct.

If, however, we were inclined to set no bounds to the

[5] The chromosome situation in the germ cells of female doves and pigeons
is as yet quite unknown. But whatever it may be, our statement illustrates
the difficulties of a chromosome theory in the cases under consideration.
We make use of a familiar case in which XY germs are male-producers,
and XX germs female producers.

[6] *I. e.*, in eggs of identical (original) chromosomal constitution.

marvels of selective power that may be exhibited by the sex chromosomes, and to feel that even the above difficult formula remains for them a possibility, we may refer to the decisive data obtained in studies on the sex behavior of the birds which are hatched from such a sex-controlled series. We shall there see that those data differentiate *several grades of females.* Some are quite nearly males, —though they lay eggs. Is it too hazardous to suggest that in one and the same egg the Y could hardly have "gone out" to allow the egg to develop into a female, and yet have "stayed in" in order to deliver the relative masculinity that we easily detect and measure? If sex is directly the creature of a sex chromosome, the sex situation found in some of my female doves requires that the male-producing chromosome be eliminated from, and retained in, one and the same egg! The only alternative that it is within my power to imagine is that in addition to the selective elimination of the Y's during autumn, there be further postulated a gradual fractional elimination of parts of this chromosome, larger and larger parts being eliminated during the progress of the season. Or, that the reverse of this occurs, namely that the Y, during the progress of the season, *gradually adds* something of X quality to itself, finally becoming more X than Y. For those who would value this interpretation I have no evidence or word of contradiction. The fact must always remain that our procedures have not only produced male and female from ova of opposed initial tendency—largely under control—but that several *grades of intermediate sex* have also been produced.[7]

[7] Three previous publications, besides several addresses before the American Society of Zoologists and elsewhere, have clearly stated this result. The publications now two years since, and the citations are as follows: (*a*) *Carnegie Year Book*, No. 12, 1913 (p. 322), Report of Year's Work. "The results strongly indicate that the hereditary basis of sex (and, therefore, probably all characters) is a quantitative, graduated thing; not qualitative and alternative as rather generally believed." (*b*) *Science*, N. S., Vol. 39, No. 1003, Mar., 1914 (p. 440), "A Quantitative Basis of Sex as Indicated by the Sex Behavior of Doves from a Sex Controlled Series." "These . . . results together with our very abundant data on the storage metabolism of the ova of these forms, and the initial fact of sex-control itself, strongly

We shall be able presently to note more closely the con-
clusive facts as to the matter of a differential matura-
tion. Continuing our examination of the further data
which we know correlate with this sort of a sex-series we
shall meet with additional and other kinds of facts which
lead toward a constructive view of the nature and basis
of sex; facts immediate and specific concerning the meas-
ured powers or capacities of these series of ova which
present us the sort of sex-series in question—facts which
reveal sex in quantitative terms.

Correlations marked (2) and (3) on Chart 1 were first
noted by Professor Whitman. I have been able every
year to find many confirmations of his conclusions.

The curve for "Developmental Energy" on the chart
indicates a progressive seasonal decrease of this capacity
in the fertilized eggs; a decrease from spring to autumn.
Now the evidence is unquestionable for the lowest part
of the curve—the autumn. In general, least develop-
ment proceeds from the last eggs of the season. These
are the *largest* eggs of the year. There is also less de-
velopment in the second eggs of the clutch. These are
the larger of the clutch. It is thus seen that the larger
the yolks the less "developmental energy" possessed by
them.

The "Length of Life" of the several offspring of such
a sex-series tells again of an advantage possessed by the
earlier hatched birds, and of a more limited life-term
affixed to the later hatches. It is further probable that
within the group of clutches giving rise to females only,
a longer average life-term falls to those who hatched
from the first egg of the clutch, than to those arising
from the second. Here, then, as in correlation no. (2)
the smaller eggs of clutch and season are the eggs pro-

indicate that the basis of sex is a fluid, reversible process; that the basis
of adult sexual difference is a *quantitative* rather than a qualitative thing."
(c) *Bulletin of the American Academy of Medicine,* Vol. 15, No. 5 (Oc-
tober, 1914) (pp. 265–285), "The Determination of Sex and Its Experi-
mental Control." "The sum of these results, together with the initial fact
of sex control itself, practically prove that the basis of sex is a fluid, re-
versible process, that the basis of adult sexual difference is a *quantitative*
rather than a *qualitative* thing (p. 277)." etc., etc.

ductive of "strength." The larger eggs both of clutch
and season more often display "weakness." And in
passing we might note that by the procedures involved
in these sex-series it is possible to graduate the fatal
dosage, and in great measure to predict which of particu-
lar germs must come to an end first.

The fourth kind of fact pertaining to the eggs of this
series, proceeds from the results of more than 800 chem-
ical analyses of individual eggs. The results of earlier
studies of this nature were described in 1911[8] and 1912
more fully than time limits will here permit; but the na-
ture of these results can be noted with the help of Chart
3. It will be observed that not only does the size of the
egg increase with its later position in the series, i. e.,
with lateness of season, but the percentage of energy-
yielding or stored materials increases as much as, or pos-
sibly more than, is indicated by the size—or net weight—
of the yolk.

The importance, for our present purpose, of the re-
sults of these analyses is that they conclusively show (1)
that the male-producing egg of the spring is an egg that
stores less material than does the female-producing egg
of the autumn. (2) That the male-producing egg of the
clutch *stores* less material than does its female-producing
mate. (3) That the eggs of old females *store* more ma-
terials, and—as has been noted—yield a higher percen-
tage of females, than do birds not old. Therefore, it be-
comes evident that the egg of female-producing tendency
is one whose storage metabolism is high, as compared
with eggs of male-producing tendency. The analyses
show that during the season successive clutches present
higher and higher storage, i. e., the earlier clutches store
less—are more male-like; the later ones all store more—
are more female-like; and as we have seen, the eggs of
the low storage period give rise to males, those of the
high storage period produce females. Here we obtain a
close view of that upon which sex difference rests. Un-

[8] Papers read before the American Society of Zoologists. For abstract,
see *Science*, N. S., Vol. 35, pp. 462–463, March 22, 1912.

mistakably, less storage and high storage pertain respectively to the male- and female-producing germs. Unmistakably, our procedure—connected with generic cross, season and overwork—delivers males from the smaller storages in the earlier eggs. Unmistakably, these procedures raise the storage in all of the later eggs, and unfailingly we then find that these eggs yield only, or almost exclusively, females. And if we eliminate the factor of wide—or generic—cross and mate the female with one of her own species, then we see that the production of males and females coincides from the first with two sizes of eggs in the clutch—males from the smaller first, female from the larger second. Only after overwork and season have raised the storage value of the eggs, is this situation, in such a mating, seriously disturbed. And the disturbance—associated with an increase in the storage metabolism of all the eggs,—delivers, as before, an excess of female offspring.

The progressive *increase in storage capacity* of the eggs during the season—under overwork—is to be interpreted as a *decrease in the oxidizing capacity* of these same eggs. Living cells in general dispose of ingested food material by storing it, or by burning it. The products of the oxidation are removable and do not serve to increase the bulk of the cell. Likewise the low-storage capacity of the male-producing eggs as compared with the high-storage capacity of female-producing eggs is therefore an index of higher oxidizing capacity of the male-producing eggs as compared with the female-producing eggs.

The fifth correlation relates to the percentage of water in the eggs of spring and autumn, and in the two eggs of the clutch. These figures for one series of analyses are given on the chart (3) last examined. They show a higher water content for the eggs of the spring (male-producers) as compared with the eggs of autumn (female-producers); indeed, each pair of eggs from the first of the season onward has a slightly higher moisture value than the pair that follows it. The analyses further show a higher percentage of water in the first egg of the clutch

than in the second in all cases.   If the results of my 800
analyses all ran as smoothly as do the 8 of this series there
would be no doubt of a perfect correlation of high mois-
ture values with small eggs, *i. e.,* with male-producing eggs
—both small eggs of season, and small eggs of individual
clutches.   But the results are not thus uniform and
smooth.   There are some series which seem seriously to
depart from the order noted above.   These can not be dis-
cussed here.   We can, however, record our own belief that
the situation represented in the chart is, in the main, in-
dicated by the moisture determinations.

Now the evidence that higher water values are asso-
ciated with male-producing eggs, lower water values with
female-producing eggs is of high importance in connec-
tion with our own generalization as to the basis of ger-
minal sex-difference; and is further of much interest as
being the means of demonstrating that in the—as I be-
lieve—several valid cases of sex-control now known, one
thing in common has really been effected, this though the
work has been carried out on a considerable variety of
animals, and though the procedures have themselves been
most various.   The thing that seems to have been effected
in all cases has been the *raising or lowering of the gen-
eral metabolism of the treated germs.*   If this conclusion
be definitely established biology may congratulate itself
that the further and complete analysis of this hereditary
character lies near at hand; is open to definite and easy
attack by methods already of demonstrated trustworth-
iness in this and other fields.   And surely if such result
is possible it is timely, now when the ''box within box''
revival has the sex character, like all others, dissociated
from all *processes* that can be studied or measured, and
associated with a *particle* so minute as hopelessly to defy
all direct and functional investigation.

That higher water values in the tissues is associated
in *development* with increased metabolism is a fact well
established.   We need cite here in reference to ''tissue
growth and repair'' only the well-known fact of the higher
water-content of embryonic tissues, and Minot's calcula-

tion that in a particular mammal 99 per cent. of growth power is lost before birth. In respect to ''heat production'' or the ''basal metabolism'' of embryo and adult the data for comparison are not extensive, but it too lends support to the view that this basal metabolism is higher in the young than in the adult. It may be added that Benedict and Emmes[9] have recently shown by very exact measurements that the basal metabolism of men is higher by about 6 per cent. than that of women.

If a higher metabolism exists in male-producing *germs,* and this is associated with higher water-content, as we concluded in 1911, it is easy to see why a number of procedures have since been shown to effect a control of the production of sex. In 1912 Miss King desiccated toads' eggs and obtained 87 per cent. of females. This was the converse of the earlier experiments of Hertwig, and of Kuschekewitch, who ''over-ripened'' frogs' eggs—a process during which they were found to *take up water*— and obtained, in the experiments of the latter author, as many as 100 per cent. of males. I think we can now see it was a shifting of the metabolism, through the agency of the water values, that produced the shifting of sex in the eggs of the frog and the toad.

More recently still, Whitney has effected a change in the sex of the offspring of the rotifer—*Hydatina*—a change from female- to male-production by means which he considers as serving to increase metabolism in the treated forms. Confirmation of Whitney's conclusion that it is a heightened metabolism that brings about male-production is now to be had in the result obtained by Dr. A. F. Shull[10] who finds that an *increased oxygen supply* leads toward an increased production of males in *Hydatina.* It now seems clear that a *heightened metabolism* in the Rotifers is the agency of increased male-production.

9 Benedict, F. G., and Emmes, L. E., ''A Comparison of the Basal Metabolism of Normal Men and Women,'' *Jour. of Biol. Chem.,* Vol. 20, No. 3, 1915.

10 Advance abstract of a paper to be presented at these meetings, December 29, 1915.

The greater production of males in cattle—indicated by Thury, Russell, and several others—from eggs that have remained unfertilized for a period of hours, is almost certainly correlated with an increased water-content which these eggs obtain before fertilization. We do not know by direct observation that the ova of the cow takes up water from the fluids that it meets in the reproductive passages. We do know that this is true for the eggs of every amphibian, reptile and bird that has been investigated. Von der Stricht has, however, described phenomena in the yolk granules of the extra-ovarian egg on one mammal—the bat—which phenomena I am quite assured from my own earlier studies on the yolk spheres, definitely indicate that in this one mammal in which the data permit a judgment, the egg does take up water from the fluid that it meets in the Fallopian tube. There is good reason to believe that the changed sex-ratios of cattle can be associated with changes in the egg-metabolism effected through, or connected with, differential water values.

The important recent work of Baltzer convincingly shows the plastic, fluid, controllable and reversible nature of sex in *Bonellia*. And, it would be difficult to believe that the larva that attaches itself to the "rüssel" of an adult, then quickly and fully differentiates, and becomes a *male*, is not displaying a higher metabolism than is the larva that rests for long in the mud and sand, and after prolonged growth becomes a *female*. Baltzer's results deserve a much more extensive statement than can be given here.

Many points, too, in Geoffrey Smith's illuminating studies on sex in the spider crabs would seem to be in harmony with the view that the castrated males progressively lose their initial advantages of a higher metabolism, and that they then become more female-like as they approach the lower metabolic levels which are normal to the females. Though Smith, so far as I am aware, has not thus interpreted his results.

The point to these citations is that sex control, in the several various forms in which it has been accomplished,

has been accomplished fundamentally by the same means
in all—a changed metabolism, in which a higher water-
content of germ and higher metabolism for male-produc-
tion, and lower water-content and decreased metabolism
for female production, have been definitely shown to be
associated in a number of instances. Whitman learned
in pigeon hybridization an additional—an entirely dif-
ferent—*means* of accomplishing the *same end* of height-
ening the metabolism of the germ. And, this additional
means definitely tends toward male-production. The
wider the cross (within the limits of the "developmental
compatibilities" of the germs) the greater the vigor and
strength added *by the mere act of crossing*—and at the
same time the more assuredly will such crosses produce
males. Even the closely related varieties used in most
Mendelian crosses have not failed to indicate the greater
vigor of the heterozygote.

A sixth series of studies has been made on size of the
parents and offspring concerned in these sex-controlled
series. Seasonal and age fluctuations in the parents, and
in both sexes of both parent species; size of offspring as
related to their sex, to season, and to the egg of clutch,
have been studied during three and one half years. We
have found no subject that presents so many complica-
tions as does the matter of the size of offspring in this
series. Only a single aspect of the matter will be treated
here. The seasonal fluctuation in size of the parents used
in the "overworked" or sex-controlled series is, how-
ever, a simple matter. Our results show—as indicated
by the lower curve on the chart (1)—that such parents
weigh most in winter and spring; least in the autumn,
reaching a minimum in August and September. In other
words, during the period when the female parent lays
her largest eggs, she herself, and her consort, are smallest
in size. I have had no charts prepared showing the sea-
sonal curves for individual birds, but data for such curves
in great number are available.

Now, the single word I wish to say on the relation of
size in the offspring to the order of the eggs of the clutch,

and as affected by the procedure of overwork, may be
more quickly said with the aid of the charts.

One chart (only Chart 2 is reproduced here) shows the
weight average of each individual hatched during the
year, from two simultaneous matings of *alba* × *risoria*.
Series I is from an older pair, previously overworked;
series II is from a younger pair, little—or not at all—
previously overworked. It will be noted that series I is
throwing large eggs, a predominance of females, and that
the *size* of the offspring—even of the males—is prevail-
ingly that of the *females* rather than the males of the
parent species.[11] Series II is throwing smaller eggs, a
nearly equal proportion of the sexes, except at the end
of the season, and the size of the offspring is decidedly
larger than in series I; and, in fact, approximates to the
size of the *males* of the parent species. In both of these
series it will be observed that size of offspring[12] is also
correlated with the order of eggs in the clutch.

For series I, we have complete data for the year pre-
ceding and the year following the term covered by the
chart. The weights for the former were: Av. for ♂'s
172 gr.; ♀'s 166 grams. For the succeeding year—early
1915—these weights are ♂'s 157 gr.; ♀'s 156 gr. Clearly,
during the three-year period a change in size of offspring
is progressively occurring; and the change runs from a
size comparable to that of the males of the parent species,
to a final size that is somewhat below that of the females
of the parent species. The egg-size was known in this
same series to have progressively and simultaneously
changed from greater male-producing tendency to a de-
cided female-producing tendency.

The seventh line of study intended to analyze the sea-
sonal and clutch deliveries of the sex-controlled series is
concerned with arrangements by which the sex-behavior
of the birds from such series is tested. In these pro-

[11] The males, in both of these species, average 10–15 grams heavier than
the females; the *risoria* birds are slightly larger on the average (5–10
grams) than *alba*.

[12] The weights given for individual birds represent the average of the
monthly, or bi-monthly weights for the year.

cedures female is mated with female, and male with male.
Such pairs—from a very few selected pairs of parents—
are kept mated for a period of six months. The three
and one half years that this study has been pursued has
enabled us—using 30 to 50 birds—to test one and the
same bird with seven others. Most of the birds used—
for lack of success with the incessantly fighting males—
have been females, and most of the seven successive tests
with each bird have been made with its sisters of the same
series. The members of the pairs are kept apart except
when under observation; when put together—as they are
twice daily—the records are taken of those females of
the pair which behave as males in copulation with their
mates. Three facts are definitely established by the data
obtained: (1) The females of the *orientalis* $\times$ *alba* cross
(they are dark in color) are more male-like in their sex
behavior than the females of the reciprocal cross (these
are white in color). (2) Females hatched from eggs laid
earlier in the season are more masculine in their sex be-
havior than are their own full sisters hatched later in
the season. *And, several grades of females can be thus
seriated according to season of hatching.* (3) The female
hatched from the first egg of the clutch is more masculine
than her sister hatched from the second of the clutch
in a great majority of the cases. And in nearly all these
latter matings the more masculine bird is so decidedly so
that she takes the part of the male a full 100 per cent. of
the time in copulating with her very feminine clutch-mate
sister.

A fuller account of this situation was given, with the
assistance of charts too large to exhibit or describe here,
before the Society of Zoologists in 1913.[13] The nature
of this behavior has been adequately recorded by means
of moving-picture films. Such records were also made
showing the reversal of the known sex-behavior of such
pairs by means of appropriate injections of ovarian and
testicular extracts. Those films were demonstrated in
this hall—or in one near-by—in connection with an ad-

[13] Abstract in *Science*, March 20, 1914.

dress before the local chapter of Sigma Xi some 20 months ago.

The injection of the extracts of gonads, performed now on the third series of birds, has resulted—quite against our wish—in the death of a number of birds. In the main the deaths from ovarian injections were of the more masculine birds; while the deaths from testicular injections have been among the more, or most, feminine birds. The numbers concerned at present are not large, and a further definite study of the matter will be made before final conclusions are drawn. But the limited data now at hand indicate that the eighth correlation listed on Chart 1 is as it is exhibited there.

A ninth, and very accurate and convincing kind of information concerning the germs involved in these sex-series has been obtained by means of the bomb calorimeter. The heat of combustion of some 200 egg-yolks has been determined. One such series of determinations for 1914, in which all available eggs were burned, is shown on Chart 4. It will there be seen that the first clutch of the season bore a higher caloric value than the second, but is otherwise the smallest of the year. Beginning with the second clutch laid in June, the succeeding clutches to December 1 bear higher and higher heat values. In all clutches too, except the very first, the second eggs show a higher storage of heat units than do the first of the clutch. Here we find the conclusions reached from studies on the weights of yolk, and on yolk analyses, fully confirmed by a study of the burning value of the materials stored. And confirmed by a method in which the error involved in the determination is wholly negligible. The most accurate method, for the study of the storage values of male- and female-producing ova, gives too the results most consistent with the breeding data.

The tenth and last of these correlations deals with embryological or morphological data. It was found that some females dead at relatively advanced ages showed persistent right ovaries. The right ovary in pigeons

normally begins degeneration at or before hatching and is wholly absent from the week-old squab. It soon became evident that the persistent ovaries were found practically exclusively in birds hatched from eggs of overworked series. Further study has shown in addition that they arise almost wholly from the eggs of autumn, and predominantly then from the second egg of the clutch— that is from eggs otherwise known to have the greatest or strongest female-producing tendency. These ovaries have sometimes weighed nearly a third as much as the adult left ovary with which they were associated, and have been found in such birds dead at all periods from a few days to fifteen months. We here attempt no adequate description of this situation, but one can not have observed the frequency of the persistence of this ovary in the birds hatched from the eggs otherwise known to be the most feminine from these overworked series without conviction that the same pressure which carries the eggs of spring from male-producing to female-producing levels, also carries the earlier female-producing level, to another yet more feminine.

In conclusion, the studies that have thus far been made on sex, and on the experimental control of sex, in pigeons go very far, we believe, toward an adequate demonstration that germs prospectively of one sex have been forced to produce an adult of the opposite sex—that germs *normally* female-producing have, under experiment, been made to develop into males; and that germs which were prospectively male-producing have been made to form female adults. That neither selective fertilization, differential maturation nor a selective elimination of ova in the ovary can account for the observed results. Further, and perhaps of more importance, these studies throw much new light on the nature of the difference between the germs of the two sexes. This difference seems to rest on modifiable metabolic levels of the germs; males arise from germs at the higher levels, females from the lower; and such basic sex differences are quantitative, rather than qualitative in kind.

THE CALCULATION OF LINKAGE INTENSITIES[1]

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Two methods of estimating the intensity of linkage are in use. One consists of crossing individuals heterozygous for two or more linked genes with homozygous recessives. This is the more direct method, because the gametic ratio—barring differential viability—is exhibited directly by the zygotic frequencies. The other method employs ordinary F_2 ratios derived from selfing F_1 or breeding together like F_1 individuals. Here the gametic ratio can only be inferred from the numerical relation of the zygotic classes. The results may be disturbed not only by differential viability, as in the first method, but also by selective fertilization, if that occurs, and may often be materially influenced by chance in random mating where the numbers are small. In fact, this method is so undesirable that it should not ordinarily be used where the other method is practicable. It is true, however, that the mechanical difficulties of crossing certain plants are so great and the number of seeds produced per flower so small that often the ordinary F_2 results are alone available. It is important, therefore, to have a means of calculating gametic ratios from F_2 zygotic numbers.

Since no direct formulæ for calculating gametic ratios from observed F_2 data have heretofore been available, the problem has been attacked in an indirect way. A series of F_2 zygotic ratios has first been calculated from a corresponding series of gametic ratios. Next the observed F_2 results have been compared with the calculated series, the closest fitting calculated ratio determined, and the corresponding gametic ratio taken as that responsible for the observed F_2 results.

The method of determining the closeness of fit between calculated and observed numbers used by Bateson, Punnett and their co-workers was mere inspection. (See Bateson and Punnett, 1911.) The unreliability of this method was pointed out by Collins (1912) who made use of Yule's coefficient of association for the same purpose. The well-known formula for this coefficient is $(ad - bc)/(ad + bc)$, where a, b, c, d are the frequencies of the phenotypic forms AB, Ab, aB, ab, respectively. From a table giving the coefficients of association for a series of gametic ratios, the best fitting gametic ratio is chosen by inspection or interpolation. This method is satisfactory except for the higher gametic ratios where slight differences in the coefficients of association correspond to wide differences in the gametic ratios. Since the same intensity of linkage gives somewhat higher coefficients of association for coupling than for repulsion, particularly for the lower linkage values where the association coefficient method is most reliable, two tables must be used.

Formulæ, by which gametic ratios can be approximated directly from F_2 data without the use of coefficients of association and without respect to whether coupling or repulsion is involved, would seem to merit trial. Such formulæ are presented later in this paper. Moreover, it is often desirable to reverse the calculation, that is, to determine zygotic frequencies from assumed gametic ratios. A single formula suggested for this purpose gives accurate results for both coupling and repulsion. This formula will be presented first because the others are developed from it.

Bateson and Punnett (1911) suggested two empirical formulæ for calculating zygotic frequencies from assumed gametic ratios, one for coupling and the other for repulsion. Neither one, of course, is applicable to both types of linkage, though both formulæ are true for independent inheritance. If A and a are allelomorphic genes and B and b are a similar allelomorphic pair—the capital letters

denoting dominance—and if $2n$ equal the sum of the gametic series,[2] then the gametic series and the phenotypic zygotic series, *AB, Ab, aB, ab,* for coupling and for repulsion are:

	Gametic Series			
	Ab :	Ab :	aB :	ab
Coupling	$n-1$:	1:	1:	$n-1$
Repulsion	1:	$n-1$:	$n-1$:	1

	Zygotic Series			
	Ab :	Ab :	aB :	ab
Coupling ..	$3n^2-(2n-1)$:	$2n-1$:	$2n-1$:	$n^2-(2n-1)$
Repulsion ..	$2n^2+1$:	n^2-1:	n^2-1:	1

That is, the formulæ of Bateson and Punnett are expressed in terms of the sum of the gametic series. But the same thing can also be expressed in terms of the several members of the gametic series. Thus, if $r:s$ is any gametic ratio, the usual form of gametic series is $r:s:s:r$ and the frequencies of the ten possible genotypic classes and of the corresponding four phenotypic classes are:

Genotypes   Phenotypes

$$
\begin{aligned}
AB \cdot AB &= r^2 \\
AB \cdot Ab &= 2rs \\
AB \cdot aB &= 2rs \\
AB \cdot ab &= 2rs \\
Ab \cdot aB &= 2s^2
\end{aligned}
\left.\right\} AB = 3r^2 + 4rs + 2s^2
$$

$$
\begin{aligned}
Ab \cdot Ab &= s^2 \\
Ab \cdot ab &= 2rs
\end{aligned}
\left.\right\} Ab = 2rs + s^2
$$

$$
\begin{aligned}
aB \cdot aB &= s^2 \\
aB \cdot ab &= 2rs
\end{aligned}
\left.\right\} aB = 2rs + s^2
$$

$$
ab \cdot ab = r^2 \quad \} \ ab = r^2
$$

The general formula for calculating a phenotypic zygotic series from a given gametic ratio is, therefore,

$$3r^2 + 2(s^2 + 2rs) : s^2 + 2rs : s^2 + 2rs : r^2 \qquad (I)$$

The sum of the zygotic series is $4r^2 + 8rs + 4s^2$ or $(2r+2s)^2$, which, when expressed as

$$(r + s + s + r)(r + s + s + s + r),$$

2 Bateson and Punnett considered n to be some power of 2, but this limitation need not apply here.

indicates how the formula is derived. Reference to the diagram will make this clear. Since r and s are any positive quantities, formula I is applicable to coupling

	r AB	s Ab	s aB	r ab
r AB	r^2 AB.AB	rs AB.Ab	rs AB.aB	r^2 AB.ab
s Ab	rs Ab.AB	s^2 Ab.Ab	s^2 Ab.aB	rs Ab.ab
s aB	rs aB.AB	s^2 aB.Ab	s^2 aB.aB	rs aB.ab
r ab	r^2 ab.AB	rs ab.Ab	rs ab.aB	r^2 ab.ab

DIAGRAM SHOWING IN TERMS OF r AND s THE NUMERICAL RELATIONS OF THE F_2 ZYGOTIC CLASSES THAT RESULT FROM COMBINATIONS OF THE GAMETIC CLASSES AB, Ab, aB, ab OCCURRING IN THE RATIO SERIES $r:s:s:r$. The dominant genes A and B are indicated by horizontal and vertical lines respectively, while their allelomorphs a and b are indicated by the absence of such lines. (See formula I.)

$(r>s)$, repulsion $(r<s)$ and to independent inheritance $(r=s)$. It, of course, gives the same result as the empirical formulæ of Bateson and Punnett, but is more convenient in that one formula takes the place of the two. It is easy to use since the fourth term of the zygotic series is the square of r, the second and third terms each the square of s plus twice the product of r and

s, and the first term the sum of the second and third plus three times the fourth.

An approximation of gametic ratios can be obtained from observed zygotic ratios by simple formulæ derived from formula I. If the actual values of $s^2 + 2rs$ could be assumed to be identical in all cases, it would follow from formula I that $4r^2 = AB + ab - (Ab + aB)$ and $r = \sqrt{(AB + ab - Ab - aB)/4}$. Similarly, $4(s^2 + 2rs) = AB + Ab + aB - 3r^2$ and $s = \sqrt{(AB + Ab + aB + r^2)/4} - r = \sqrt{(AB + Ab + aB + ab)/4} - r$. If E is the sum of the extreme terms and M the sum of the middle terms of the observed zygotic series, the formulæ for approximating gametic ratios are, then,[3]

$$r = .5\sqrt{E - M}$$
$$s = .5\sqrt{E + M} - r \qquad\qquad \text{(II)}$$

If it is desired to compare the observed F_2 frequencies with a calculated series of frequencies, the procedure, obviously, is to calculate the gametic ratio by formulæ II —or by means of the coefficient of association—and then to calculate the zygotic series by formula I—or by one of the two formulæ of Bateson and Punnett. This procedure is not always necessary, however, for a theoretical zygotic series can usually be readily computed directly from the observed frequencies. If AB, Ab, aB, ab is the series to be calculated from the observed frequencies, it follows from formulæ I and II that

$$Ab = aB = M/2$$
$$ab = (E - M)/4 \qquad\qquad \text{(III)}$$
$$AB = M + 3ab$$

Since a zygotic series calculated in this way necessarily meets the conditions imposed by formula I, the gametic ratio can be approximated from it more readily than from the observed frequencies. Since by formulæ I and II

$ab = r^2$ and $s = .5\sqrt{E + M} - r$,

$$r = \sqrt{ab}$$
$$s = .5\sqrt{E + M} - \sqrt{ab} \qquad\qquad \text{(IV)}$$

[3] Since r and s are necessarily positive, negative roots are disregarded.

Formulæ IV are not to be used in connection with observed F_2 frequencies except when the latter approximate closely the form demanded by formula I, that is, when the first term of the observed frequencies equals approximately the sum of the second and third terms plus three times the fourth term.

In cases of repulsion, where the fourth term of the zygotic series is always relatively small and, therefore, where the first term should be only slightly greater than the sum of the second and third terms, it may happen that the sum of the first and fourth terms, E, is actually less than the sum of the second and third terms, M. In such cases, formulæ II (and consequently formulæ III and IV also) can not be employed, for, if E is less than M the quantity under the radical ($E - M$) is negative and has no real root. In such cases, the gametic ratio must be calculated by means of the coefficient of association.

The method here suggested for calculating gametic ratios from observed frequencies never gives quite the same results as that obtained by the association-coefficient method except when the observed series approaches closely the form demanded by formula I. Naturally, then, the more widely the observed frequencies depart from this form the greater the difference between the results given by the two methods. Since the coefficient of association gives reliable results if the tables to be used with it are based upon sufficiently small differences in the gametic ratios employed in its preparation, it follows that the methods proposed in this paper give only approximate results. It is also true, therefore, that the nearer the observed frequencies approach the form of formula I, the closer the approximation obtained by formulæ II (or III and IV).

The two methods have been applied to numerous cases taken from published accounts of linkage studies and the goodness of fit tested by the method suggested by Harris (1912). The differences, $o - c$, between the observed frequencies, o, and the calculated frequencies, c, of the sev-

eral classes are determined and $S[(o-c)^2/c] = x^2$ calculated, S indicating summation.

With n, the number of classes, here equaling four, and x^2, the probability, P, that departures from the calculated series as great as those observed might occur through the errors of random sampling, is obtained by reference to Elderton's (1901) table (see also Pearson, 1914). Wherever appreciably different gametic ratios have been obtained by the two methods, P has been found to be greater for the association-coefficient method than for the method based on formulæ II. The former method has, therefore, given the closer fit. Since, in most of the cases to which the test has been applied, x^2 is less than one and since such values are not listed in Elderton's table, x^2 has been used directly for the comparison of the two methods. Where n is constant, the larger x^2 the less the probability.

While this test for goodness of fit has shown the association-coefficient method to be the better of the two, the fact that in most cases x^2 was less than one for both methods indicates that the approximate method suggested here ordinarily gives results such that the departures of observed from calculated frequencies might well be due to errors of random sampling. The method has been found convenient and usually sufficiently accurate where only an approximate determination of the gametic ratio is desired. Where the observed frequencies depart widely from the form given by formula I, this method should not be used. It should be noted, however, that in such cases no calculated series fits the observed results well. This limitation to the use of the new method does not lessen materially the convenience of using it where it is applicable. By a mere inspection of the observed frequencies, it can usually be told whether they conform fairly closely to formula I, that is, whether the first term is approximately equal to the sum of the second and third plus three times the fourth.

A few examples will illustrate the use of the approximate method of calculating gametic ratios from observed

data and afford a means of comparing it with the association-coefficient method.

Harris (1912) has quoted an example of coupling in sweet peas from the studies of Bateson, Saunders, and Punnett[4] and calculated P where the gametic ratios are taken as $7:1$ and $15:1$, the only ratios considered in the original paper. The phenotypic classes are based on shape of pollen and color of flowers and the observed frequencies are purple long 493, purple round 25, red long 25, red round 138, total 681. As determined by Harris, on the basis of a $7:1$ gametic ratio, $P = .0053$ or $x^2 = 12.7699$. On the $15:1$ basis, $P = .3086$ or $x^2 = 3.6375$. The chances against the $7:1$ ratio are, therefore, 199 to 1 and against the $15:1$ ratio about 2 to 1. For this same material, Collins (1912), using the association-coefficient method—Coef. Assoc. $= .982 \pm .004$—naturally suggested a $12:1$ gametic ratio—Coef. Assoc. also $= .982$—and pointed out the fact that the deviation from the $7:1$ ratio is 9 times and from the $15:1$ ratio about twice the probable error. By formulæ III, the calculated series becomes $485.75 + 25.0 + 25.0 + 145.25 = 681$. By formulæ IV, $r = 12.052$ and $s = .996$ or a gametic ratio of $12.1:1$. The $12:1$ ratio obtained by the association-coefficient method gives a zygotic series of $485.5 + 25.2 + 25.2 + 145.1 = 681$. Both methods, then, give gametic ratios approximately the same and practically identical zygotic series, namely, $485 + 25 + 25 + 145$. On the basis of this series, $x^2 = .4387$ and P is so large that it is useless to determine it. In short, both methods give gametic ratios that fit the observed data extremely well.

The next example of coupling presents a very different condition. It has been quoted by Bridges (1914) from Punnett's (1913) summary of reduplication series in sweet peas. The phenotypic classes are based upon sterility of anthers and form of flowers and the observed frequencies are fertile normal 165, fertile cretin 58, sterile normal 58, sterile cretin 78, total 359. It can be seen at a glance that these frequencies are far from

[4] Rept. Evol. Com., 4: 11.

what formula I demands—$58 + 58 + 3(78) = 350$, over twice 165—and that therefore the approximate method can not be depended upon in calculating the gametic ratio. It is interesting to note, however, just how unreliable it is in comparison with the association-coefficient method. By formulæ III and IV, the calculated zygotic series becomes $211 + 58 + 58 + 32 = 359$, $r = 5.6$, $s = 3.8$, and the gametic ratio is approximately $1.5 : 1$. Bridges referred the case to a $2 : 1$ ratio (Coef. Assoc. $= .558$), though the coefficient of association is .588 which is equivalent to a gametic ratio of $2.1 : 1$ (Coef. Assoc. $= .586$). Punnett compared the observed frequencies with a series derived from an assumed $3 : 1$ ratio. The zygotic series calculated from these ratios are, for the $2 : 1$ ratio, $219 + 50 + 50 + 40 = 359$; for the $2.1 : 1$ ratio, $220 + 49 + 49 + 41 = 359$; and for the $3 : 1$ ratio, $230 + 39 + 39 + 51 = 359$. If now the criterion of goodness of fit be applied to the four calculated series the values of x^2 are, for the $1.5 : 1$ ratio 76.1, for the $2 : 1$ ratio 52.0, for the $2.1 : 1$ ratio 51.4, and for the $3 : 1$ ratio 51.3. Values of x^2 above 30 are not listed in Elderton's table, but where $x^2 = 30$ and $n = 4$, $P = .000,001$, which means that there is only one chance in a hundred thousand of deviations so great as the observed ones being due to the errors of random sampling. Where neither of the two methods of calculating the zygotic series gives a better fit than in this case, it is immaterial which fit is the worse.

As an example of repulsion, the same characters, in sweet peas may be used. The observed frequencies (Bateson and Punnett, 1911) are $336 + 150 + 143 + 11 = 640$. Bateson and Punnett assumed that the gametic ratio concerned was $1 : 3$. The coefficient of association is $-.706$, which is equivalent to a gametic ratio of $1 : 2.74$. By formulæ II–I or III–IV, a ratio of $1 : 2.45$ is indicated. The values of x^2 are for the $1 : 2.45$ ratio .649, for the $1 : 2.74$ ratio .302, and for the $1 : 3$ ratio .536. Here again the association-coefficient method gives the better fit, but the probability is great that the deviations of the observed from the calculated frequencies, even in case of

the approximate method, might be due to errors of random sampling.

As an illustration of the fact that the approximate method can not be used in some cases of repulsion, even when the observed frequencies fit fairly well the series calculated by the association-coefficient method, an example of linkage between dark axils and fertile anthers in sweet peas quoted from Punnett by Bridges (1914) may be taken. The observed frequencies are $1335 + 643 + 714 + 2 = 2694$. The value of r can not be determined by formulæ II nor by III and IV, because $1335 + 2 - (643 + 714)$ is a negative quantity (-20) and has no real root. The coefficient of association is $-.988$, which is equivalent to a gametic ratio of $1:17$, though Bridges assumed a ratio of $1:20$. On the basis of this $1:20$ ratio, $x^2 = 5.68$ and $P = .1309$. On the basis of the $1:17$ ratio, $x^2 = 4.04$ and $P = .2615$, or odds of about 3 to 1 against the occurrence of deviations as great as those observed.

It may be said, then, that the formulæ suggested here afford a convenient method of approximating gametic ratios from zygotic series, when the observed frequencies are in fair accord with a series based on formula I—or the formulæ of Bateson and Punnett. When the observed frequencies are far from this type no method gives a close fit between observed and calculated results.

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THE MECHANISM OF CROSSING-OVER. IV

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THE "map" of the first chromosome, based on these experiments, is shown below:
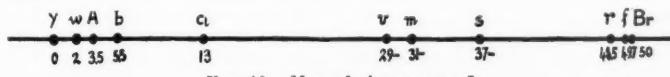
FIG. 10. Map of chromosome I.

The figures represent the distances of the factors from yellow, the first one in the line, and are calculated merely by adding together the intermediate distances. This map gives almost exactly the same proportionate distances between the different loci as does that obtained by combining the results of linkage experiments performed by other workers, in which usually the inheritance of only two or three factors was followed at one time. Each set of ratios, therefore, confirms the accuracy of the other. The absolute distances in the present map are, however, somewhat shorter, being $\frac{6}{7}$ the length of those in the composite map. This was caused mainly by the comparatively large number of non-cross-overs produced by a few females; in the rest, the crossing-over frequencies were about normal. It may, therefore, be concluded that chromosomes which differ in regard to eleven pairs of factors behave in the same way, so far as crossing-over is concerned, as those which are alike except for two factors. This is contrary to a suggestion made by Punnett. Moreover, the fact that chromosomes differing in so many factors behave normally is here especially noteworthy, because 11 of the 12 recessive factors were in the same chromosome.

The results of the experiments with the second chromosome may now be tabulated. 462 offspring of females

heterozygous for the ten mutant factors used in this group have been recorded. The table only gives the result with respect to nine characters, however, as arc wing was not followed in all of the experiments. (The data given later as to its position have, accordingly, not been calculated from quite as large a count of flies as have the data for the other factors.)

CLASSIFICATION OF FACTOR COMBINATIONS TRANSMITTED BY FEMALES HAVING THE COMPOSITION: $\dfrac{S_{tr}\ b_l\ p_u\ v_g\ a_r\ s_p}{d_a j\quad c_v\quad b_a}$

	Streak		Not Streak		Total
Non-cross-overs					
	Sbpvas	68	*djcb*	82	150
Between	*Single Cross-overs*				
S_{tr} and d_a.................	*S d j cb_a*	11	*b pv s*	15	26
d_a and b_l....................	*S j cb_a*	24	*db pv s*	19	43
b_l and j......................	*S bj cb_a*	1?	*d pv s*	0	1?
j and p_u......................	*S b cb_a*	3	*d jpv s*	6	9
p_u and v_g...................	*S b p cb_a*	14	*d j v s*	20	34
v_g and c_v...................	*S b pv cb_a*	10	*d j s*	11	21
c_v and s_p...................	*S b pv b_a*	51	*d j cs*	50	101
s_p and b_a...................	*S b pv sb_a*	0	*d j c*	0	0
Double Cross-overs					
S_{tr} and d_a; p_u and v_g........	*Sdj v s*	2	*b p cb_a*	1	3
S_{tr} and d_a; v_g and c_v........	*Sdj s*	1	*b pvcb_a*	0	1
S_{tr} and d_a; c_v and s_p........	*Sdj cs*	5	*b pv b_a*	8	13
d_a and b_l; j and p_u...........	*S j pv s*	1	*db cb_a*	0	1
d_a and b_l; p_u and v_g..........	*S j v s*	5	*db p cb_a*	5	10
d_a and b_l; v_g and c_v..........	*S j s*	5	*db pvcb_a*	1	6
d_a and b_l; c_v and s_p..........	*S j cs*	5	*db pv b_a*	8	13
j and p_u; p_u and v_g...........	*S b v s*	1	*d jp cb_a*	1	2
j and p_u; v_g and c_v...........	*S b s*	0	*d jpvcb_a*	1	1
j and p_u; c_v and s_p...........	*S b cs*	7	*d jpv b_a*	1	8
p_u and v_g; v_g and c_v.........	*S bp s*	0	*d j vcb_a*	2	2
p_u and v_g; c_v and s_p.........	*S bp cs*	3	*d j v b_a*	3	6
v_g and c_v; c_v and s_p..........	*S bpvcs*	2	*d j b_a*	1	3
Triple Cross-overs					
S_{tr} and d_a; d_a and b_l; c_v and s_p .	*Sd bpvb_a*	1	....		..
S_{tr} and d_a; j and p_u; c_v and s_p ..	....		*b cs*	1	..
d_a and b_l; j and p_u; p_u and v_g ..	*S j pcb_a*	1	....		..
d_a and b_l; j and p_u; c_v and s_p..	....		*db cs*	1	..
d_a and b_l; p_u and v_g; c_v and s_p..	....		*db p cs*	1	..
j and p_u; p_u and v_g; c_v and s_p...	....		*d jp cs*	1	..

Total Single, Double, and Triple Crossing-over

Between	Observed Number	Per Cent. of Crossing-over
S_{tr} and d_a	45	9.7
d_a and b_1	77	16.7
b_1 and j	1?	0.2?
j and p_u	25	5.4
p_u and v_g	59	12.8
v_g and c_v	34	7.1
c_v and s_p	150	32.5
s_p and b_a	0	0.0

In the case of this chromosome, too, the law of linear linkage is graphically illustrated by the characteristic "sectional" mode of interchange between the groups. The non-cross-overs here constitute only 32.5 per cent. of the population, whereas the single cross-overs make up 51.1 per cent., the double cross-overs 15.2 per cent., and the triple cross-overs 1.3 per cent. In making a map of this chromosome, the chances of error are greater than in the preceding case, since not so many flies have been obtained. Nevertheless, the values correspond very closely with estimates of the results obtained in other work, although figures exactly representing the sum total of other work are not just now available for comparison.
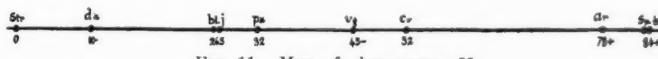
FIG. 11.   Map of chromosome II.

Let us now construct a curve showing the frequency with which, in the experiment with the first chromosome, points various distances apart showed coincidence of crossing-over. Suppose that in this curve the horizontal line represents the distance apart of the two coincident cross-overs, and the vertical line the per cent. of cases in which double crossings-over at such distances occur. For example, if it were known that double crossing-over for a distance anywhere between 15 and 16 units occurred in .2 per cent. of all cases the height of the curve above the figures 15 and 16 would be made .2 vertical units. Now, each case of double crossing-over that actually happens

among the 712 flies obtained for group I must represent $\frac{1}{712}$, or .14 per cent., of all the cases. If, then, a crossing-over is found to occur somewhere between c_1 and v, and one occurs coincidentally between s and r, the two points of crossing-over may have been as far apart as c_1 and r (36), or as close together as v and s (8), or at any intermediate distance. Therefore we have no right to make this case stand, in the curve, for a coincidence that happened at a particular distance (say 10–11) and to raise the ordinates for this particular distance by .14 units. Each distance between 8 and 36 is consequently given partial credit in our curve for the occurrence of this coincidence, and so each of the 28 ordinates between 8 and 36 is raised to an average height of $\frac{.14}{28} = .005$ approximately. All the other cases are treated in a similar way, and thus the curve shown by the heavy line in Fig. 12 is obtained.
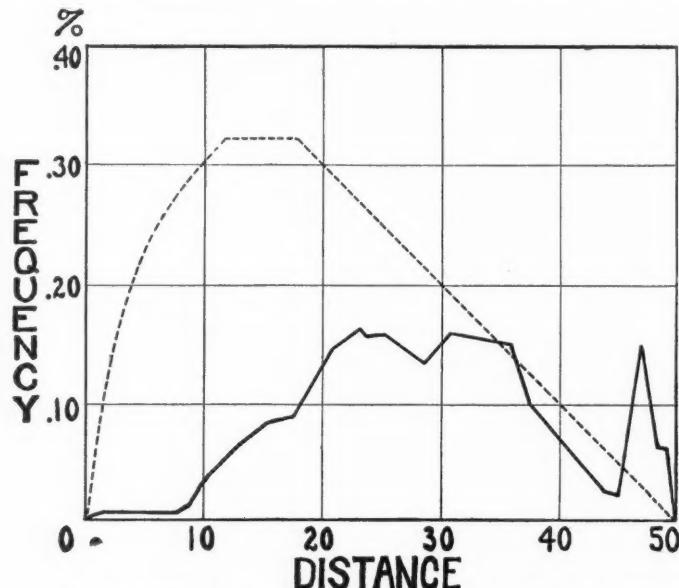
Fig. 12. Curve showing the observed frequency of double crossing-over in chromosome I, for points various distances apart. The dotted line shows the frequency expected on pure chance.

But although these ordinates are, on the *average*, raised by this amount, each one is not raised equally, for there is less chance that double cross-overs should have the most extreme possible values than medium values. The total addition of .14 units to the curve should hence be distributed among the different possible ordinates according to the relative probabilities that the two points of crossing-over should have been the distance apart represented by these respective ordinates. These various probabilities for the different ordinates, in the case of any specific double cross-over, may be represented in the form of a curve, and the main curve of double cross-over frequency shown in figure 12 is thus really a composite in which these individual curves for each double cross-over have been added together. We may now consider the way in which the individual curves of probability are calculated.

Let us take the case of the double cross-over that occurred between c_1 and v and coincidently between s and r. We have already calculated that the distance between the two points of crossing-over must be somewhere between 8 units and 36 units (see second paragraph above). The curve for this individual double cross-over will therefore start at 8 on the abscissa and continue to 36. What height shall it have along the ordinates between these points? Let the region c_1 — v be divided into 8 equal parts —abcdefgh—of two units each, as shown below.

```
13                      29           37              49
c₁   — 16  units —       v — 8 un.—  s    — 12   un. —   r
|  |  |  |  |  |  |  |  |             |                |
|                       |            |                |
|  |  |  |  |  |  |  |                |                |
  a  b  c  d  e  f  g  h
```
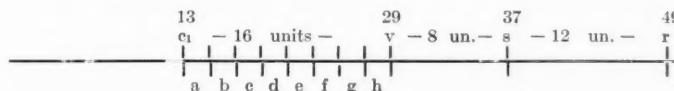
It will be seen that a double cross-over of 8 to 10 units length (*i. e.*, having 8 to 10 units between its two points of crossing-over) which passes between the factors c_1 and v, must go between them in the region h, if its other point of crossing-over is to be between s and r. However, any double cross-over of 10 to 12 units length which passes through either g or h will also pass between s and r, and so there is twice as much chance for double cross-overs of

this length to occur as for those 8 to 10 units long. Similarly, those 12–14 units long may be three times as numerous, for they may pass through f, g, or h, and so with each increment of length, up to 20, there will be an equal additional amount of chance for a double cross-over of that length (passing through the required sections, $c_1 - v$ and $s - r$) to occur. Thus our curve of probability rises in regular steps from 8 to 20; if we could have divided the distance $c_1 - v$ into an infinite number of parts, instead of into 8, these steps would each be infinitely small, and so we should have a straight line rising from 8 to 20.

Beyond this point the rise in probability ceases; a double cross-over between 22 and 24 units long has no more chance of happening than one of 20–22 units. Reference to the figure will show that a double cross-over of 20–22 units passing through any of the regions from c through h will separate s from r and thus fulfill the requirements, but a double cross-over 22–24 units long, while it has the additional alternative of passing through b, can not pass through h without its second point of crossing-over falling to the right of section $s - r$. Similarly, one 24–26 long may not pass through g or h, though it may pass through any region from a to f; double cross-overs of all these lengths therefore have the same chance of occurring, and our curve along the corresponding ordinates would hence be a horizontal line.

Double cross-overs longer than this would have less and less chance of occurring; one 26–28 long could only pass through regions $a - e$, one 28–30 only through $a - d$, and so the curve falls again in a straight line to the zero level at 36.

The same rules can be shown to apply to all cases: the curve starts at a place on the abscissa representing the distance apart of the innermost factors involved (in the above case this distance was $v - s$, $= 8$); it rises in a straight line for a distance equal to the length of the smaller section involved (above, this was the distance $s - r$, $= 12$, so that the line rose to point $8 + 12$, $= 20$);

it then proceeds horizontally until a distance from the starting point of the curve equal to the length of the longer section has been passed (above, this was the section $c_1 - v, = 16$; thus the line proceeded on a level to point $8 + 16 = 24^6$); then it falls in a straight line to a point on the abscissa representing the distance between the outermost factors involved (above, the distance is $c_1 - r, = 36$). The height to which the curve rose is determined by the fact that its area (the sum of all the ordinates) must have a value representing the per cent. of total cases in which such a double cross-over occurred (above, each double cross-over must have a curve with an area $= .14$, since each fly was .14 per cent. of the total count).

It will be noted that for each individual curve the probability is calculated on a basis of pure chance, no account being taken of possible interference, which, if present, would tend to make the longer distances more likely than the shorter, and so to raise the right end of the curve at the expense of the left. In other words, each *individual* curve represents the frequency with which double cross-overs of different lengths would happen *within* the particular regions dealt with (in our case above, regions $c_1 - v$ and $s - r$), if there were no interference and they had a purely chance distribution, within these regions. The *composite* curve thus errs rather by showing too little effect of interference than too much. All interference which it does show—that is, all deviation between it and a curve representing an entirely random distribution of double cross-overs—must then be due solely to the way in which the double cross-overs were found to be distributed *among* the various regions, as no assumption of interference was made in calculating out the curve for each double cross-over.

The curve representing the proportion of double crossovers of different lengths which would have been found on an entirely random distribution (no interference) is

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[6] The discrepancy between this figure (24) and that (26) found by the method of trial used above would disappear if the region $c_1 - v$ had been divided infinitely instead of only into eight parts.

shown by the dotted line. To make comparison with the other curve legitimate, it had to be constructed by the same method,—namely, by making a composite of individual curves, each of which represented the probabilities for a certain type of double cross-over—only, instead of using the observed numbers of double cross-overs of the different types, in constructing it, it was necessary to use the numbers of double cross-overs of the different types that would have been observed if there had been no interference. (This curve hence represents the results of a chance distribution both among and within the various regions.) In the case of each type of double cross-over, the way to find the per cent. of individuals showing it that would be produced if there were no interference, is to multiply the total per cent. of crossing-over in the first region by the per cent. in the second region, as explained in section 4a. (Thus, the per cent. of double cross-overs passing between A and B and between C and D equals per cent. of cross-overs between A and B times per cent. of cross-overs between C and D.) This per cent., then multiplied by the total number of individuals counted, gives the number of such double cross-overs theoretically to be expected in the absence of interference. When such calcu-lations for each different possible kind of double cross-over have been made, and the individual curve for each then made, the latter may be combined to form a composite curve like the curve shown by the dotted line.

The end desired is of course to compare the dotted and the heavy-lined curves and see what proportion of the double cross-overs various distances apart, that were expected on pure chance, actually occurred. Therefore a new curve (Fig. 13) may be made, representing this *relative coinci-dence, i. e.,* the per cent. which each frequency on the observed curve formed of each frequency on the expected curve (see sect. IVa). This curve consequently shows the rise or fall of the index with which we are already famil-iar, and which we have called simply "coincidence."

Owing to the fact that not very large figures have so far

been obtained, we must be cautious about accepting the exact values shown in the curve of coincidence; this applies not so much to the main portion of the curve as to the right-hand end (shown in dotted lines), for in the case of very long double cross-overs, very few kinds are even theoretically possible, compared to the number of different
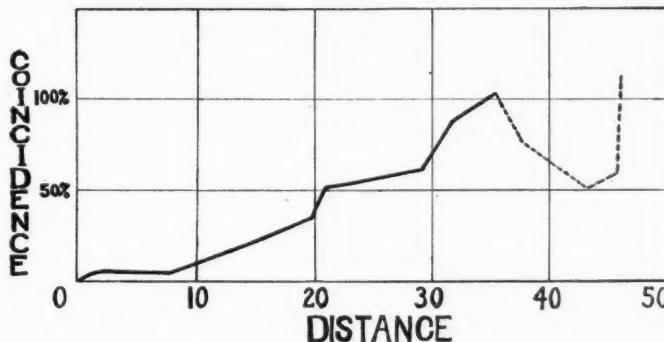

FIG. 13. Curve of coincidence for chromosome I, *i. e.*, the ratio of observed double crossing-over for points in chromosome I various distances apart to double crossing-over expected on a chance basis.

positions in which short double cross-overs of a given length may be found. Accordingly, the marked fall, followed by great rise at the very end of the first curve has no true significance.

Certain points may be seen to stand out plainly, however. It is clearly evident that interference is great for short distances—*i. e.*, that relative coincidence is low; as distance increases the coincidence rises, at first, quickly, but beyond a certain point the rise ceases.

There is no indication of a usual length of loop of less than half the length of the chromosome, as cytological observations on strepsinema stages would suggest, and as would therefore be expected on the view that crossing-over occurs at that stage. The fall seen near the right hand end is entirely unreliable, as has been explained. But, even if taken at its face value, the drop at this point can have no significance for the question at issue, for a fall due to the loop would have to be as long as the whole pre-

vious rise.   In addition, the curve should, on this explana-
tion, rise high above the 100 per cent. level at its modal
point, whereas it is evident that, so far as the significant
figures go, it does not rise much above 100 per cent. at any
point.   It would be premature, however, to generalize fur-
ther on these results.

The curve for group II will not be presented until
greater numbers of flies have been recorded.   It may be
stated, however, that this curve too shows the phenomenon
of interference, although, since the factors are not so close
together, the crossing-over for rather small distances can-
not so well be followed.

The great variability possible in the distance between
two points of crossing-over is shown not only in the above
curves, but may be graphically illustrated from a single
case.   This fly was the triple cross-over in the first chromo-
some, which has already been mentioned.   Its mother was
one of the tested females of the count, whose composition
proved to have been $\frac{\text{ywAbvmsrf}}{\text{B}_r}$, and it itself was a male
with the factors yrB$_r$.   Crossing-over, therefore, must have
taken place between y and w, s and r, and r and f.   The
minimum possible distance between the first two points of
crossing-over is 42, the maximum distance between the
second two is 14.   The latter is the smallest distance ever
observed between two points of crossing-over.   It may
here be mentioned that it will be of great interest, when
more extensive figures are obtained, to see whether in the
second chromosome the same coincidence holds between
crossings-over on opposite sides of the middle point as
between crossings-over an equal distance apart, but on the
same side.   The bend of the chromosomes in the middle, or
some other structural difference here due to the attach-
ment of the spindle fiber at this point, might cause the re-
sults to be different in the above two cases.

Incidentally, the results demonstrate another point,
lying in a somewhat different field of genetics.   By fol-
lowing the method of keeping stocks constantly in heter-

ozygous condition, twenty-two factors have been continually outcrossed, in each successive generation, to their allelomorphs. Yet after about seventy-five generations of outcrossing, these characters do not show the slightest contamination. The experiment therefore forms an extensive test and verification of the "purity of Mendelian segregation." Castle has, however, raised the point that in determining whether characters change, we should not be content with casual inspection. One of the characters in the above experiment—dachs legs—lends itself readily to quantitative work, since one of its main features is a shortening of the tarsus and metatarsus. Measurements of the legs of about a dozen of these dachs flies, derived from the stock which had been subjected to continual outcrossing, were therefore made, as well as measurements of the legs of some dachs flies derived from a stock which had been kept pure; the values for normal flies were determined also. At the same time the thorax length of the flies was observed, in order that any difference in leg length due merely to variation in the size of the whole animal might be allowed for. The results for each individual are shown in the following table. Measurements are given in eyepiece micrometer divisions, each of which represented .026 mm.

In order to discover whether the character had become more variable as a result of outcrossing, the standard deviation of the ratios of foot to thorax, in the two stocks of dachs, was calculated from the above data. In the outcrossed stock the standard deviation was found to be .036, and in the original stock .035; that is, so far as these results can show, the variability of dachs after outcrossing has remained just the same. However this may be, the fact remains that the character, after being subjected to such long-continued outcrossing, had not approached one whit nearer to the type of its allelomorph. The slight difference in the other direction observed between it and the original mutant stock is of no significance, since just about as great differences in thorax length occurred between the

two stocks, but in opposite directions in the two sexes. The judgment based upon measurements accordingly confirms the judgments based upon inspection.

FEMALES

Dachs from Outcrossed Stock		*Dachs from Uncrossed Stock*		*Wild Flies*	
Length of Thorax	Length of Foot (Tarsus Plus (Metatarsus)	Thorax	Foot	Thorax	Foot
32 19		32 20		41 .... 31.5	
35 20		33 21		42 31	
35 20		34 19		42 31	
36 .... 19.5		35 20		42 31	
36 20		35 .... 20.5		43 32	
36 .... 20.5		35 21		43 33	
				44 34	
Averages:					
35 .... 19.8		34 .... 20.25		42.4 .... 31.9	
Ratio of foot to thorax length: .567		.596		.752	

MALES

Dachs, Outcrossed		*Dachs, Uncrossed*		*Wild*	
26 18		28.5 .... 17.5			
28 19		29 .... 19.5		26 24	
29 20		29 .... 20.5		29 26	
30 21		30 19		29 26	
31 .... 17.5		32 22		32 28	
31 19		32 .... 23.5		33 29	
32 20		33.5 23		37 32	
Averages:					
29.6 .... 19.2		30.6 .... 20.7		31 .... 27.5	
Ratio of foot to thorax: .650		.677		.887	

SUMMARY

1. Recent results complete the parallelism between factor groups and chromosomes in *Drosophila*. This strengthens the evidence that separation of linked factors is due to an *interchange between chromosomes*.

2. The chief gaps in the information regarding the total frequency of interchange in the different groups have been filled, and it is found that the usual total frequencies of separation correspond to the lengths of the chromosomes. This constitutes specific evidence that *crossing-over is the method of interchange* between the chromosomes, and that

the frequency of crossing-over between factors is determined by their distance apart in the chromosome. It supplements the other evidence for these conclusions that had previously been found by Sturtevant in the linear manner of linkage of the factors.

3. It seems uncertain whether crossing-over occurs in the strepsinema stage, as concluded by Janssens, or earlier in synapsis. The cytological evidence at present at hand would seem insufficient to settle this point. Possible tests for various alternative mechanisms of crossing-over are proposed.

4. In order to study the nature of crossing-over by means of "interference," stocks were made up that differed in regard to many factors. Females heterozygous for 22 pairs of factors were thus obtained, and a special method was devised for testing their output. Other special methods for obtaining multiple stocks, and for eliminating discrepancies due to differential viability, have also been presented.

5. The results have been arranged in the form of a curve showing the amount of interference for various distances. The results thus far obtained confirm those obtained by less exact methods, and also give evidence that interference decreases gradually with distance from a point of crossing-over; this, taken together with certain evidence from non-disjunction, lends some probability to the view that crossing-over occurs at an early stage in synapsis.

6. A case of crossing-over in an embryonic cell of a male is reported.

7. Incidentally, the experiments have afforded an extensive test of Castle's assumption of contamination of factors by their allelomorphs. Outcrossing in each generation for 75 generations has failed to change any of the factors.

The author is deeply indebted to Professor Morgan, and wishes also to convey his appreciation of the active cooperation so often rendered him by E. R. Altenburg and

A. H. Sturtevant, who, moreover, on several occasions helped to tide the stocks over critical periods during which it was not possible for the author to carry on the work. Thanks are also due to C. B. Bridges, for supplying several multiple stocks as well as for the use of a number of mutants which he had already located but an account of which he has not yet published.

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SHORTER ARTICLES AND DISCUSSION

DISTRIBUTION OF THE CACTI WITH ESPECIAL REFERENCE TO THE RÔLE PLAYED BY THE ROOT RESPONSE TO SOIL TEMPERATURE AND SOIL MOISTURE

As is very well known, it is the common habit, when referring to the relation of a ''plant'' to its environment, to mean the subaerial portion only, leaving quite to one side the subterranean parts. That there is little logic in this will be readily acknowledged, although the possible causes are not far to seek. In the first place, for patent reasons, roots do not greatly excite our admiration or curiosity, and thus have received little attention in the field. Further, relatively little experimental work has been done on the roots of plants other than on seedlings and growing in solutions. And besides these conditions which refer immediately to the plant, there is a nearly related one which has to do with its environment, especially with the root environment. The soils and the soil condition of whatever sort are probably more difficult to study, and the results more difficult to express in a manner capable of ready application than the subaerial environment of the plant. However, it has not been its difficulty alone that has been the deterrent in the study of the environment of roots since certain features, for instance the soil temperature, can be easily learned by appropriate apparatus. Could we have a comprehensive series of data touching this feature alone, to mention no other, we should be in possession of a very useful engine for use in comparative studies on causes underlying the distribution of plants, and, further, through it the study of the root-systems of plants, and of their biological value, would be greatly stimulated.

While it is here recognized that the presence of a plant in its environment is an expression of the response of the whole plant to the entire environment, it is necessary, for the purpose in hand, to ignore the responses of the shoots, and to focus our attention for the time on the root relation alone. It can be noted, however, as is very well known, that the activities of the latter may be reflected in those of the former. Such a condition, having inter-

esting possibilities, was observed at the Coastal Laboratory, at
Carmel, California, and may be briefly referred to in this place.
Among the species growing in the experimental plots at the labo-
ratory are *Opuntia versicolor* and *Fouquieria splendens* from the
vicinity of the Desert Laboratory, Tucson, Arizona. Owing to
the usual low temperature of the air, and soil, these species gen-
erally make little or no shoot growth at Carmel. When, however,
the roots of the plants are kept in soil whose temperature is
25–30° C., the shoots remaining in the cool air, not only do the
roots grow rapidly, but new shoots and fresh leaves are promptly
formed. Without pursuing this phase of the matter further it can
be seen that analogous results might occur in nature should the
soil conditions, for instance its color or the relation of the soil
surface to the incident heat rays,[1] be such as to bring about a
relatively warm soil environment. Under such conditions it is
clear that only a study of the soil temperatures, and the responses
of the roots to soil temperatures, would provide the key to the
solution of the shoot behavior and to all of its accompanying
results.

It is generally recognized that the soil acts as a reservoir for
heat, and that the daily course of soil temperature is unlike that
of the air immediately above it. Thus, the roots are subjected to
temperature conditions which are quite different from those
affecting the shoot of the same organisms. The shoot is warmer
by day and colder by night than the root and it is improbable
whether the roots of most woody plants are often subject to
"optimum" temperature conditions, as must frequently be the
case of the shoots. An exception to this statement, however, is
to be found in the cacti where the most favorable soil tempera-
tures are of great importance among those environmental features
that may be called definitive. The roots of most cacti of the
Tucson region, and possibly elsewhere, lie near the surface of the
ground. For the most part they are less than 30 cm. deep.
Inasmuch as the rate of root growth of the cacti, as will be shown
below, is relatively slow at temperatures much under the
"optimum," the importance to these plants of a shallow position
of the roots will be apparent. It is only in the upper soil horizon
that such favoring temperatures are to be found. It is of inter-

[1] Cannon, W. A., "On the Relation of Root Growth and Development to
the Temperature and Aeration of the Soil," *American Journal of Botany*,
Vol. 2, p. 211, 1915.

est to note, on the other hand, that deeply placed root-systems, such as of *Prosopis velutina,* may have a relatively rapid growth rate at relatively low temperatures.[2] In such a case it is quite possible that the rôle played by root response to temperature in species distribution may be less important, or, at any rate, different from that played by the roots of the cacti, for example, to the distribution of members of that family.

We will now glance at the most striking conditions of soil temperature as they obtain at the Desert Laboratory, where much of the work here referred to has been carried on, before taking up a résumé of the response of the roots of the cacti to the temperature of the soil and the relation this suggests to the general distribution of the family.

Three series of soil thermographic records, which are now being supplemented by others, have been kept at the Desert Laboratory. These relate to three depths, namely, 15 cm., 30 cm., and about 2.6 m. Although the records cover a series of years, it will serve the purpose in hand if we refer to those for the year 1910 only.

The mean maxima and the mean minima temperatures for the three depths will provide sufficient data for interesting comparisons.

At the shallowest depth, 15 cm., the mean maxima temperatures for midwinter and midsummer were 8.1° and 34° C., respectively. The mean minima, for the same seasons, were 3.9° and 30.8° C. At a depth of 30 cm. the maximal range was from 12.2° C., in January, to 33° C., in July, and the minima temperatures, for the same months, 10° and 32.2° C., respectively. It was observed that from June to September, inclusive, the curve of the mean maxima for this depth did not fall below 32.2° C.

At a depth of 2.6 m., the mean maxima temperatures ranged from 18.6° C., in January, to 27° C., in July.

Upon comparing, in a general way, the mean maxima for the different soil depths we see that the shallowest soil is the warmest from April to August, inclusive; that in September and October only the highest temperatures are found at a depth of 30 cm.; and that in late winter-early spring the lowest level is also the warmest.

The relation of the rate of root growth in *Opuntia versicolor,* as representative of the cacti, to different soil temperatures indicates interesting conditions and possibilities, and will be given in the following paragraph:

2 Cannon, W. A., *l. c.*

Very many experimental cultures, of various kinds, made both at the Desert Laboratory and the Coastal Laboratory, have shown that the growth rate of the roots of *Opuntia,* within limits, varies directly with the temperature. It is relatively slow at 20° C., and most rapid at 34° C. The hourly increase in length of the roots at 20° C. is about 0.3 mm., and at 30° C. it is approximately twice this. Above 34° C., the rate falls off rapidly and ceases at about 42.5° C. Below 20° C., the growth rate is very slow, as, for example, at a temperature of about 16° C. an increase in length of a perfectly normal root was found to be only 1 mm. in 14 hours. The maximum rate, taking place at about 34° C., is about 1 mm. an hour.

Referring back now to the soil temperatures, it will be seen that the roots of this species are exposed to optimum conditions in July and August only, although the soil temperatures for one month before and one month following this period, at a depth of 30 cm., or less, is also high enough for an effective growth rate. The soil temperatures, at this depth, in the other months, and at the lowest level throughout the year, are not sufficiently high for the best root activity. However this may be, we find, in short, that suitable soil temperatures obtain at the depths occupied by the roots of the cacti during four months of the year. But it does not follow that root growth goes on throughout this period for the reason that the foresummer is arid and the shallow soils are impossibly dry, having less than 10 per cent. of moisture. Active root growth of the cacti, in fact, commences with the coming of the summer rainy season, about the middle of July. It is ended by the cooling of the soil in early autumn. The length of the active growing season of the roots of the cacti, therefore, does not usually exceed six or eight weeks.

It is in the response of the roots to the temperature and moisture conditions, as just sketched, that lies the crux of the suggestion offered in this paper, namely, that conditions being otherwise favorable, the cacti, which are shallowly rooted, occur in such regions as have the superficial soils moist at the same time they are suitably warm, and they are wanting where such soil conditions fail.

With the reaction of the roots of the cacti to temperature in mind, it will be instructive to examine briefly the leading climatic features, so far as they affect the case in point, of the regions in which the cacti form a conspicuous portion of the vegetation.

According to Engler and Prantl, the cacti occur mainly in the

dry parts of Mexico, in the portions of the United States which border on Mexico, in eastern and central Brazil, and in portions of the Andes countries. Taking two or three genera as examples, we learn, for instance, that *Cereus* occurs in Mexico, and in the Andes of Argentina and Brazil. *Echinocactus* extends from the southwestern part of our country to Brazil and Chili *Opuntia* is found in Mexico, Peru, Chili, in Central America and in the southwestern portions, especially, of the United States. Although certain species are outside of this range, as especially certain opuntias, where the winters are exceedingly cold, all are subject in summer, when active growth takes place, to conditions which are in rather close accord. A glance at the summer climates of these regions will, I think, establish this point.

In the central part of Mexico, at Tehuacan, the annual rainfall is about 15 inches, most of which occurs in summer, and at Pueblo, 70 miles distant, and at a higher altitude, where the annual precipitation is more than twice that at Tehuacan, 72 per cent. of the rain comes in the warm season. The Tehuacan region has been characterized as being the richest of any known in cacti.[3] At Chihuahua, where the rainfall is 10.86 inches, the amount falling in the summer season is also over 70 per cent.

In the southwestern part of the United States, where the cacti constitute a conspicuous portion of the flora, a relatively large summer rainfall is also reported. At Tucson, for example, the precipitation amounts to 11.74 inches annually, of which 54.7 per cent. is received in July, August, and the first part of September.

Turning now to South America, and without especial regard as to the presence of cacti at the particular stations quoted, we find that over a relatively large area, a large percentage of rainfall is in the warm part of the year. For example, at Matto Grosso, Brazil, the greatest rainfall is in December. From June to August and generally for a month before and after this period, the climate is usually dry.[4]

Along the east coast rain occurs from February to April, June to September being dry. In the Cordilleras of Bolivia and Peru, the rainy period is in December–March, and the climate is dry from April to October. At La Paz, although rain may fall any month of the year, December to February is regarded as being the season of rain.

[3] MacDougal, D. T., "Botanical Features of the North American Deserts," Carnegie Inst. Wash. Pub. 99, 1908.

[4] Hann, "Handbuch der Klimatologie," Bd. II, 1910.

We have supplemental evidence that the cacti grow most suc-
cessfully in such warm temperate moderately arid regions as have
precipitation in the warm season from the work of the Australian
commission for the study of certain species which have escaped
from cultivation in several countries, especially Australia, and
have become a pest.[5]   In Queensland and New South Wales
species of *Opuntia* constitute a serious weed.   At Westward and
Rockhampton, Queensland, where the cacti are particularly a
nuisance, over 50 per cent. of the annual rainfall occurs in
December–March, inclusive.   Soil temperature data from Bris-
bane, depth one foot, show that the mean temperature from
October to April is between 22.7° and 27.9° C., and that during
the colder portion of the year the mean temperature at that depth
is below 20° C.[6]

The commission studied the cactus problem in several different
portions of the world, among which were Cape Colony, central
and southern India, southeastern and southern South America
and the Mediterranean region.   It will be instructive to sketch the
leading climatic features of definite localities where cacti were
found to have escaped cultivation.

In southern Africa, species of *Opuntia* occur in a naturalized
condition in the Great Karoo and in the Transvaal.   In parts of
the former region, as at Graaf Reinet, the species are abundant.
At Graaf Reinet, according to Knox,[7] where the total precipita-
tion is 15.29 inches, 63 per cent. occurs in November–March.   In
the Transvaal, where the escaped cacti are less numerous, the
rainfall is 26.94 inches, of which 81 per cent. occurs in November–
March.

In northern Africa the cacti escape from the oases very little,
and the same is to a degree true of other portions of the Mediter-
ranean region.   In Algeria and Tunis, according to Knox, the
rains are almost exclusively restricted to the winter season.

In India species are naturalized over a large territory, as, for
example, in the Madras Province and in the Panjab.   In Madras
the prickly-pear has become a formidable evil throughout several
districts.   At Madras[8] 79 per cent. of the total precipitation takes
place in August–September.   In the state of Mysore, also, the

[5] Report of the Prickly-pear Traveling Commission, Brisbane, 1914.
[6] "Results of Rainfall Observations made in Queensland," H. A. Hand, 1914.
[7] "The Climate of the Continent of Africa," 1911.
[8] Hann, "Handbuch der Klimatologie," *l. c.*

opuntia is commoon. At Mysore, according to Hann, 81 per cent.
of the rainfall is from May to October. At Lahore the prickly-
pear is not so abundant as further south, but it occurs escaped,
nevertheless. Here the July–August rains comprise 55 per cent.
of the total annual precipitation.

In South America the Commission examined naturalized opun-
tias in portions of Brazil and Argentina chiefly. An important
prickly-pear region is northwestern Argentina, where native as
well as introduced species of cacti occur in abundance. At Salta
there is as good as no rain in the cold season, between May and
September. At Tucuman, 69 per cent. of the rainfall takes place
between December and March, inclusive (Hann), and at Cata-
marca, between November and March, inclusive, 81 per cent. of
the total annual precipitation occurs.

Without pursuing this phase of the matter further, it would
appear, in short, that in regions where cacti are abundant, either
native or introduced, rains occur during the warm season. It is
not intended to discuss in this place the actual amount of rain-
fall which falling in the warm season makes the presence of a
cactus flora possible. It is well known, however, that the amount
of precipitation in regions where cacti occur is extremely unlike,
and that it may vary from season to season in any one region.
This last, in fact, is one of the leading characteristics of an arid,
or semi-arid region. So far as regards the precipitation differ-
ences in separate regions frequented by cacti, it is interesting to
note that at Rockhampton, Queensland, it is 40.09 inches,[9] while
at Phoenix, Arizona, it is 7.06 inches,[10] and that in the former
region 20 inches occurs in the warm season, while the amount of
summer precipitation at Phoenix is between 0.9 and 2.1 inches,
as means of the extremes.[11]

In the Mohave the annual rainfall is 4.97 inches,[12] about two
inches less than the mean precipitation for Phoenix. In the Mo-
have, however, 86 per cent. of the rainfall is in winter, which
greatly emphasizes the differences in summer aridity of these
regions, and points to a probable reason why cacti are almost

[9] "Results of Rainfall Observations made in Queensland," H. A. Hunt,
l. c.

[10] "Botanical Features of North American Deserts," D. T. MacDougal,
p. 95, 1908.

[11] "Climatology of the United States," A. J. Henry, U. S. Dep. Ag. Bull.
Q., 1906.

[12] MacDougal, *l. c.*

wholly wanting in the flora of the latter region. From these climatic facts it appears that while soil moisture is a condition *sine qua non* of the presence of the cacti, the range of the actual amount of soil moisture must be very great indeed, so, in short, it results that the temperature is the factor in direct control, thus a very important limiting factor.

Should we sum up, therefore, the factors thus far mentioned as being important among those which determine the distribution of the cacti, we find, in the first place, that the shallowly placed root-system subjects the roots to the greatest possible extremes in soil temperatures, including those that are high, and, at the same time, makes it possible for the plants to advantage from the minimum effective rainfall. Further, an effective growth rate of the roots takes place only at relatively high soil temperatures. And, finally, a certain but highly variable amount of moisture must be present in the soil. Since the *crux* of the matter, however, appears to be the fact that the root-system of the cacti are essentially superficial, there is the additional factor, or factors, which bring about this circumstance. These are at present unproved, but the results of experimental studies, not published, indicate that among them must be included the response to the oxygen supply of the soil.

<div align="right">W. A. Cannon</div>

Desert Laboratory.

THE INHERITANCE OF CONGENITAL CATARACT

In the February number of the American Naturalist there is an article from the Bussey Institution by Jones and Mason[1] in which an attempt is made to show that congenital cataract behaves in heredity as a simple Mendelian recessive. The authors from a study of family histories published by Harman in the "Treasury of Human Inheritance" come to conclusions at variance with those of Bateson and Davenport, which authors they are perhaps unjustly disposed to criticize. The paper is well written and embodies a considerable mass of data, so that the reader not familiar with this particular problem might easily be led to think that the older investigators had really made a mistake in interpretation. The evidence, however, does not seem to

[1] Jones, D. F., and Mason, S. L., "Inheritance of Congenital Cataract," The American Naturalist, Vol. L, No. 590, pp. 119–126, February, 1916.

warrant such a conclusion, as the present paper will attempt to demonstrate.

It is stated on page 120 of the article in question that the data used in the paper are taken from the tables accompanying Harman's publication. Since we are concerned wholly with a question of interpretation we may confine ourselves to these tables.[2] The families recorded in the tables are classified by Jones and Mason as follows (p. 120):

After discarding all the doubtful cases, and picking a sibship with its parents from the table as a family, there is left a total of one hundred and twenty-five families which are classified into three different categories, as follows: (A) Both parents normal with at least one abnormal child; (B) one parent normal, the other affected with some form of congenital cataract, with at least one abnormal child; (C) both parents abnormal, giving only abnormal children.

In each of these groups ($A, B,$ and C) it is thought that evidence is found in support of their contention that congenital cataract is a recessive character. We may now consider this evidence in the order in which it is presented.

In group A, 31 families are cited in which both parents are normal with one or more affected children. This is the strongest, or really the *only*, evidence that is offered in favor of the recessive character view. Let us examine it more closely. On going over Harman's tables, we find that of these 31 families there are 16 in which the affected individuals produced no offspring or nothing but normals. We do not wish to lay great emphasis on this point, but in such cases one should bear in mind the common clinical belief and the experimental proof (for rabbits and guinea-pigs)[3] that a certain number of congenital cataracts are produced by intrauterine poisoning without necessarily any reference to heredity.

Another possible explanation for some of the examples in group A is that they represent cases of origin *de novo*. Jones and Mason say:

[2] Harman, N. Bishop, ''Congenital Cataract,'' in the ''Treasury of Human Inheritance,'' Part IV, Section XIII *a*. Eugenics Laboratory Memoirs, XI, pp. 126–169. Pl. XXVIII–XXXIII. London, 1910.

[3] Pagenstecher, H. E. ''Uber eine Methode der gemeinsamen experimentellen Erzeugung von Augenmissbildungen und von angeborenen Staren bei Wirbeltieren,'' *Münch. Med. Woch.*, 58 Jahrg., No. 32, pp. 1716–1717. Aug. 8, 1911. (Reviewed in most of the eye journals.)

Surely it is not possible to explain so many cases as *origin de novo* or as due to faulty classification of the parents.

With reference to the origin *de novo* of characters it may be recalled that one does not have to search the literature long to find instances of the same mutation occurring repeatedly in different stocks and at different times, or of certain stocks that seem to be especially prone to mutation.[4] Congenital cataracts occur in many races of man and in other mammals. So far as the writer is aware we are not at present in a position to state, either on the basis of observed data or from *à priori* consideration just how frequently mutations may occur in the human germplasm.

Again, since Jones and Mason elsewhere in the same paper (p. 124) use the argument that "heterozygous individuals sometimes show the recessive character," we might, if necessary, use the same argument to prove the dominance of cataract. On the assumption that congenital cataract is dominant instead of recessive it might be maintained that in those cases where both parents of affected individuals seem to be normal, one of them is, after all, heterozygous, and affected children are therefore to be expected.

Finally it should be recalled that in their statistical study of these 31 families Jones and Mason do not get the results that their hypothesis demands. After having made the proper mathematical corrections there still remains a discrepancy which they do not adequately explain, the agreement between theoretical and observed results being only .418 (p. 122). In order to test what one should expect from the examination of such data when the character is recessive, I have taken a paper by Usher[5] on retinitis pigmentosa and summarized the charts in the same way that Jones and Mason summarize those of Harman. Now retinitis pigmentosa probably *is* a recessive character as is commonly believed. In the charts of Usher are recorded 44 families in which

[4] To cite a single case, we may mention the results of Barfurth in breeding fowls. In normally 4-toed races polydactylism occasionally arises *de novo*, but once having appeared is transmitted as a Mendelian dominant. Barfurth, Dietrich, "Experimentelle Untersuchung über die Vererbung der Hyperdactylie bei Hühnern. V. Mittelung: Weitere Ergebnisse und Versuch ihrer Deutung nach den Mendelschen Regeln," *Arch. f. Entwichlungsmech. d. Organism.*, Bd. 40, pp. 279–309, 1914.

[5] Usher, C. H., "On the Inheritance of Retinitis Pigmentosa with Notes of Cases," The Royal London Ophthalmic Hospital Reports, Vol. 19, pp. 130–236. 1914.

neither parent of the affected individual shows the defect (*i. e.*, both are presumably heterozygous). These 44 pairs of parents are recorded as having 320 children, of whom 77 are affected—24 + per cent. as compared with an expectation of 25 per cent. If the data on cataract were to yield results as close as this we would be more disposed to credit the view that the character is recessive.

So far as the 31 families of category *A* are concerned it must be admitted that absolute proof of the fallacy of the recessive character view can not be furnished, but it will be apparent that there is considerable evidence which not only fails to support this view, but actually points decidedly against it. This fact, taken in connection with the positive refutation which the data in categories *B* and *C* supply, makes a very strong case against the view that congenital cataract is a recessive character.

In the second category (*B*) where one parent is affected the other normal, Jones and Mason remark that "the number of affected children would be expected to be approximately the same whether the character was inherited as a dominant or a recessive" (p. 121). But it must be borne in mind that the offspring of a recessive show the 1 : 1 ratio *only* when the mate is heterozygous, and in their second table Jones and Mason assume that the parents of the children in group *B* represent the cross "Nn × nn." The question is not raised as to the probability of the occurrence of such matings nor does there seem to have been an attempt made to trace the offspring from the normal and affected members of the F_1 generation. In other words, the data of really critical significance do not seem to have been considered. As it stands, then, Table II seems to present no evidence either for or against the above hypothesis, a point which the authors themselves recognize as the quotation indicates.

Since the authors have not tabulated the data which would seem to be of most significance, we may return to Harman's original charts assuming for purposes of the discussion that congenital cataract really is a recessive. On this assumption there are two important conditions which we should expect to find fulfilled.

1. If congenital cataract were a recessive, a cataractous person married to a normal should in most cases produce only normal children. This will be apparent when it is recalled that con-

genital cataract is so rare (perhaps 1 in 4,000 or 5,000)[6] that the number of heterozygous individuals in the general population must be relatively low—theoretically not more than $1:30$.[7]   In other words, if congenital cataract were recessive the chances that an affected individual in marrying would get a heterozygous partner and thereby be able to produce affected children would be only one in thirty and the chances that the same thing would happen in several generations in direct descent *as occurs repeatedly in the charts* (the case in over 40 *different family trees*) become extremely remote.   We should not then expect families with one cataractous parent to contain affected children more often than in the above proportion.[8]

2. If congenital cataract were recessive the normal children of a cataractous parent should themselves produce affected children in half as many cases as do their cataractous sibs and the total number of affected children produced should be one half as great in the first case as in the second.   This expectation follows from the assumption that the original (grandparental) mating was $Nn \times nn$.   As a result of such a mating the F_1 generation can be composed only of Nn and nn individuals.   Neither of these should produce affected children except when married to an Nn (or an nn), and the chances of such a marriage are as great (or as remote) in the one case as in the other.   In other words, an equal number of heterozygous and pure recessive individuals of the F_1 generation should get heterozygous mates.   In these few families the expectation for F_2 would then be of a $1:3$ ratio for the $Nn \times Nn$ matings and a $1:1$ ratio for the $Nn \times nn$, which would obviously give one half as many affected children in the first case as in the second.

Harman's charts afford sufficient data to settle these points conclusively.   In regard to the first point there are 96 cases in which the cataractous child of a cataractous parent has himself

reached maturity and produced one or more normal offspring, thus proving on the assumption that cataract is recessive, that his consort was either NN or Nn. Instead of finding, as we should expect on the assumption that cataract is a recessive, that only 3 or 4 (1 : 30) of these individuals would find an Nn mate and therefore be capable of giving some affected children, what we really do find is that of these 96 families, 83 have produced cataractous children—86 per cent. It is significant, incidentally, that of the remaining 13, ten are families of three children or less. A more striking refutation of the assumption could hardly be found.

In regard to the second point we find that there are 47 normal individuals in the same F_1 generation from the supposedly Nn $\times$ nn matings who have also come to maturity and produced children. In 42 of these families only normal children have resulted. In 10 per cent. of such families, however, one or more cataractous children have been produced. But the relation between 10 per cent. on the one hand and 86 per cent. on the other is very far from being the relation of one to two.[9]

These considerations based on the results from such matings as are included in category *B*, and the F_2 descendants of such matings, furnish convincing evidence that congenital cataract is not a Mendelian recessive.

Finally as to the last point, which concerns category *C*, we can agree with the authors when they say:

> The critical test as to whether or not congenital cataract can be considered as a simple recessive character lies in the matings of abnormal by abnormal. Families of this kind should have only abnormal children. Only three such matings are available.

I find two of these, but have searched the charts in vain for the third which must be an error or inadvertently drawn from outside sources. This really is immaterial since it would require many such cases to prove the hypothesis, where a single bona fide case in which two affected individuals produce normal offspring is sufficient to overthrow it. One of the three cases cited is such.

[9] How we are to explain the five individuals in this group who have produced cataractous offspring is not material to the present consideration. Not to mention the possibility of faulty classification, possible mutation etc., they might, for instance, belong to the class mentioned by Jones and Mason in which the parent while really heterozygous appears in the recessive form.

In chart 342, III–28 and III–37 are shown as a pair of parents both of whom are affected in both eyes. The descriptions quoted from Nettleship by Harman (*op. cit.*, p. 148) show that the diagnosis is evidently based on ophthalmoscopic examinations. This is a clear case of abnormal by abnormal, and if we were to regard it as "doubtful" we could find equal justification for so regarding any other chart in the whole series. The offspring of this marriage are seven children, of whom two have cataract, three thought to have been free from it died in infancy, and two are definitely known to be normal. This is the one critical case that is needed and, taken at its face value, it completely refutes the argument for the recessive nature of congenital cataract.

In conclusion, the writer does not wish to insist on arguments from a few particular cases, nor does he wish to make purely academic distinctions in the treatment of data. In particular, he does not wish to be understood as maintaining that congenital cataract behaves strictly as a single dominant unit character— a view to which he does not subscribe. The point upon which he does insist, however, is that the view, presented in the paper under discussion, namely that congenital cataract is due to a single recessive character, not only fails to find support in the data which was presented, but is in reality actually disproved by that data.

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